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MECHANISMS OF MICROWAVE INDUCED DAMAGE IN BIOLOGIC MATERIALS

ANNUAL/FINAL REPORT

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Concerns over the possibility that exposure to electromagnetic fields can have adverse health consequences has prompted research into the mechanism of interaction between electromagnetic fields and living cells. A six-year study at the Catholic University of American, the research effort has included experimental and theoretical studies that attempt (1) to firmly establish whether or not there are, in fact, any athermal effects on living cells that can be attributed to exposure to electromagnetic fields; (2) to determine and explain the dose-response relationship between bio-effects and EM field parameters; and (3) to discover how biologic cells can detect very weak ambient fields which are much smaller than intrinsic EM noise fields always present. In this work we report that significant contributions have been made to each of the three areas described above.

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MECHANISMS OF MICROWAVE INDUCED EFFECTS IN BIOLOGIC MATERIALS

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Summary

SUMMARY

Concerns over the possibility that exposure to electromagnetic fields can have adverse health consequences has prompted research into the mechanism of interaction between electromagnetic fields and living cells. A six-year study at the Catholic University of America the research effort has included experimental and theoretical studies that attempt (1) to firmly establish whether or not there are, in fact, *any athermal effects* on living cells that can be attributed to exposure to electromagnetic fields; (2) to determine and explain the dose-response relationship between bio-effects and EM field parameters; and (3) to discover how biologic cells can detect very weak ambient fields which are much smaller than intrinsic EM noise fields always present. In this work we report that significant contributions have been made to each of the three areas described above.

We have established unambiguously that exposing living cells to electromagnetic fields can produce a significant modification of cell function. Previous experimental studies purporting to show effects on biological systems that have been exposed to weak electromagnetic fields have been criticized on the basis that the results have not been independently replicated. We have performed extensive *in-vitro* studies utilizing human lymphoma cells, human leukemia cells, and mouse cells. We have used various biological markers such as concentrations c-myc and total mRNA, and the activity of the enzyme ornithine decarboxylase. Hundreds of replication studies both within our laboratory as well as with other laboratories have been made. The data demonstrate both intra-laboratory and inter-laboratory reproducibility of results and leave no doubt that exposure to low level extremely low frequency (ELF) and ELF-modulated microwave fields can affect cellular function.

Our in-vivo experiments unequivocally confirm that low level electromagnetic fields can cause developmental abnormalities in early stages of chick embryo development. In studies of the effects of ELF EMFs on developing embryos, we have now accumulated results on over 3000 exposed chick embryos (with accompanying unexposed controls). Up to a three-fold increase in the abnormality rate has been observed in embryos exposed to weak electromagnetic fields. The lack of reproducibility of results that a number of research groups have documented in the literature has been shown to derive from control variations in the specimens tested.

Our chick embryo data, indicate that only those embryos with a genetic predisposition (approximately 20%-25% of the total) display a developmental sensitivity to electromagnetic fields.

We have demonstrated that exposure to ELF fields affects the biochemistry in developing chick embryos. In unexposed embryos, peaks in ornithine decarboxylase (ODC) activity occur after 15 hours of development (gastrulation) and after about 23 hours of development (neurulation). In the presence of weak ELF fields, these ODC peaks increase in magnitude and shift toward earlier times.

We have developed a dynamical mathematical model that describes the transient time evolution of biosystem responses to applied fields. When biological systems are exposed continuously to fields that are switched on at some initial instant, the biochemical response initially rises at a rate determined by the impressed field strength, reaches a maximum level (also field strength dependent) and subsequently decays to a steady-state value that can be lower than the basal level. We have shown that the major effect of the electromagnetic field is the enhancement of both the production and degradation rates of certain intermediate reaction products (e.g., mRNA) in the sequential chain of biochemical reactions occurring in the cell. This finding has enabled us to understand many of the unusual dose-response characteristics that have been reported in the bioelectromagnetic literature.

Our theoretical model has explained phenomena such as "power windows" (i.e. maxima in the bioresponse regarded as a function of the impressed field strength). These "windows" are shown to be natural consequences of the transient nature of the biological response. The unusual results that have been observed when various exposure schedules have been tested are understood in terms of the EM field's effect on biochemical reaction rates. Scheduling exposures for the therapeutic use of EM fields can be guided by our new understanding of the dynamic response of biological systems to those fields.

Our theoretical model can explain many of the confusing correlations (such as with wire codes) found in epidemiological studies.

We have discovered that applied electromagnetic fields can cause bioeffects only if they are temporally coherent. Exogenous EMFs must exhibit temporal coherence for times of the order of at least 5 seconds or so if bio-effects are to occur. Variation in the amplitude or frequency on time scales of less than about one second completely eliminate any effects. Conversely, if the time scale of the changes exceeds about ten seconds, the full EMF-induced effect is observed; the cells respond exactly as they would to steady-state external field conditions.

Our results indicate a new form of biological cooperativity related to the fact that electromagnetic fields can cause bio-effects only if they are spatially coherent. The data indicate that cells discriminate against thermally generated noise fields at their membrane surfaces through biological cooperativity--a relatively large number of receptor proteins must be simultaneously and coherently activated if signal transduction is to occur. This insight enables us to address the very fundamental question that has long plagued the field of bioelectromagnetism: how is it possible that a cell, existing in an electromagnetically "noisy" environment, can be affected by exogenous fields that are many orders of magnitude weaker than the local fluctuating endogenous fields? Biological cooperativity imposes on the exciting field the requirement of spatial coherence over distances encompassing many receptors so that coincident detection at several locations on the cell membrane is achieved. Thus random thermal noise neither produces an effect nor masks the effects of weak (spatially coherent) exogenous fields because it lacks the spatial coherence property that cells require if they are to respond to the field.

An important implication of this discovery of the role of spatial coherence involves its exploitation in developing techniques to render the electromagnetic fields innocuous, thereby eliminating any possible adverse health effects. Like all physical detection systems, cells are subject to the limitations occasioned by the presence of noise. This suggests that if a *spatially coherent* but temporally random noise field were superimposed on the coherent external EMF signal, then at some value of the signal/noise amplitude ratio, the discrimination mechanism would be defeated and the observed field-induced bioeffect(s) would be suppressed. Experimental studies on ODC activity in cell cultures and on morphological abnormalities and ODC activity in developing chick embryos have yielded results that support this idea.

I. In vitro Experimental Measurements to Determine Biological Response to Applied Electromagnetic Fields

a. Assessment of Biological Response to Applied Fields.

A considerable controversy surrounds the question as to whether applied microwave and extremely low frequency (ELF) electromagnetic fields (EMF) result in significant alterations of biological activity. Several factors account for such controversy. Some reported work has been poorly designed and, therefore, results from it are questionable. Other work has shown dramatic effects (1,2), but verification of these results via replication of the work in other laboratories has proven difficult, or has not been attempted. Finally, despite published reports of biological responses to EMF, theoretical arguments have been advanced in which it is claimed that any response to very weak fields is impossible (3).

Three very fundamental questions, thus, had to be approached in the initiation of our research program: 1. Is there a significant biological response to applied weak EMF?; 2. By what means do EMF and cells interact?; and, 3. Is a biological response to weak EMF theoretically possible? Experiments designed in conjunction with investigators from the Physics and Electrical Engineering groups were begun to probe these questions.

Mammalian cell cultures were selected as target systems to examine effects of EMF exposure. Cell cultures are consistent and readily manipulable biological systems which avoid many of the problems associated with animal work. Assessment of general culture parameters, such as growth rate, viability and plating efficiency, provides good, general indicators of cell health. Beyond such characteristics, however, the wide range of more specific molecular and biochemical assays is readily applied to culture systems, and cell lines are available which provide some of the differentiated functions associated with intact tissues. Further, use of culture systems obviates many of the problems associated with the accurate determination of dosimetry for exposures of intact animals.

Five cell lines were employed for the work described below. The murine fibroblast line L929 was chosen as a well characterized, non-differentiated cell type. IMR-90, a human fibroblast, was selected as a cell which displays both growth-related senescence and density-dependent inhibition of growth. Another human fibroblast, clone 12BE derived from skin tissue of a patient with xeroderma pigmentosum, was used for some experiments. This cell line exhibits deficiencies in DNA repair mechanisms. Two other human cell lines, derived from malignant tumors, were also employed. These were the HL-60 line, derived from a promyelocytic leukemia, and Daudi cells, derived from a patient with Burkitt's lymphoma.

REFERENCES Ia:

1.   Goodman, R., L. X. Wei, J. C. Xu, and A. Henderson. 1989. *Exposure of Human Cells to Low-Frequency Electromagnetic Fields Results in Quantitative Changes in Transcripts*. <u>Biochimica Et Biophysica Acta</u> 1009: 216-220.

2.   Wei, L. X., R. Goodman, and A. Henderson. 1990. *Changes in levels of c-myc and histone H2B following exposure of cells to low-frequency sinusoidal electromagnetic fields: evidence for a window effect*. <u>Bioelectromagnetics</u> 11: 269-272.

3.   Adair, R.K. 1991. <u>Phys. Rev.</u> A43:1039-1048.

b. Response of Cultured Cells to Microwave Exposure: Initial Results

Effects of microwave exposure were first assessed by examining cultures for growth rate, viability and plating efficiency after cultures were removed from the microwave field. The basic idea, which reflects the approach often taken in toxicological research, was that any serious insult to cell metabolism would be manifested in alterations to these very general indices of cell health. Cells were exposed both as suspension cultures, in a wave guide system (2.45 GHz; SARs from 5 to 1277 mW/g), and as monolayer cultures in a Crawford cell (915 MHz; SARs of 1 and 3 mW/g) for intervals ranging from 30 m to 6 h. Detailed descriptions of the exposure systems and dosimetry are provided in the Electrical Engineering sections of this report. Additional experiments involved high energy pulsed, 1.25 GHz microwaves, and microwave exposures concurrent with elevated temperatures (39 to 42°). Under none of these exposure conditions were any significant variations in cell growth or survival observed.

From such data it became clear that the general cell parameters we had selected, most of which had to be measured many hours after the termination of exposure, were unsuitable. Accordingly, more specific parameters were sought that could be examined immediately following exposure. Among several assays evaluated, enzyme activities proved useful. The first attempt at enzyme assay involved L929 cells and activities of two enzymes involved in cellular response to interferon. It was found that exposure of cells to 2.45 GHz microwaves at an SAR of 130 mW/g for 4 h produced an approx. 20% increase in activity of the enzyme 2-5A-Dependent RNase, but no change in activity of the enzyme 2'-5'-oligoadenylate synthetase (1). This limited, but significant change in the activity of one enzyme, but not in that of a related enzyme, prompted additional investigation of enzyme systems. Work on ornithine decarboxylase, described below, provided a useful indicator for determining EMF response.

REFERENCES Ib:

1. *Krause, D., J. M. Mullins, L. M. Penafiel, R. Meister, and R. M. Nardone. 1991. Microwave Exposure Alters the Expression of 2-5A-Dependent RNase. Radiatio127:*

c. Amplitude Modulated Microwaves and Extremely Low Frequency ELF Electromagnetic Fields Alter the Activity of Ornithine Decarboxylase in L929 Cells

SUMMARY

Sixty Hz amplitude modulated (AM) but not continuous wave microwaves produced a transient doubling of ornithine decarboxylase (ODC) activity in cultures of L929 cells. Other AM frequencies tested were not effective in inducing increased ODC activity. Magnetic fields at 60 Hz also produced transient doublings of ODC activity, but displayed a shorter time course. The AM microwave effect, since it requires modulation at an extremely low frequency (ELF) appears to be an ELF effect requiring demodulation of the microwave signal.

INTRODUCTION:

Ornithine decarboxylase (ODC), a critical enzyme in the production of polyamines and, therefore, an essential factor for cell proliferation (1), was selected for examination of microwave-induced effects. ODC was considered an attractive marker since its activity is readily changed by a variety of factors, including several ligands which bind to cell surface receptors. Further, work by Byus *et al.* had shown enhancement of ODC activity as the result of exposure of cultured cells both to 16 Hz amplitude modulated (AM) microwaves (2), and to 60 Hz ELF electric fields (3).

We set out to verify the microwave sensitivity of ODC activity, the goal being to provide a reliable marker of EMF exposure for use in a variety of experiments. Results of this work demonstrated a two-fold enhancement of ODC activity following exposure of cultures to either 60 Hz amplitude modulated, but not continuous wave, microwave fields and to 60 Hz sinusoidal magnetic fields.

METHODS:

Logarithmically growing cultures of murine L929 cells were maintained in Eagle's minimum essential culture medium with 5% fetal bovine serum. Daudi cells were grown in RPMI 1640 medium supplemented with 20% fetal bovine serum. Cells were plated 24 h prior to microwave exposure. To avoid serum stimulation of ODC activity, the culture medium was not changed before experiments were begun. For microwave exposure four 25 cm^2 flasks of cells were placed into a Crawford cell housed in a 37° incubator. Matched control flasks were housed outside the Crawford cell in the same incubator chamber. Cells were exposed to 915 MHz microwaves for periods ranging from 2 to 24 h, with SARs of 0.6 and 3.0 mW/g. Amplitude modulation (23%) was at

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6, 16, 60 or 600 Hz. Exposure to sinusoidal 60 Hz magnetic fields was accomplished by placing cultures between vertically oriented Helmholtz coils. Matched control cultures were placed in an identical, but separate, incubator chamber. Both 60 Hz-exposed and control cultures were shielded from stray magnetic fields by mu metal shields. At the end of exposure cells were harvested by gentle scraping, washed with phosphate buffered saline and stored as frozen pellets. Ornithine decarboxylase activities were assayed by the procedure of Seely and Pegg (4), modified by addition of 0.2% Nonidet P-40, 50 μg/ml leupeptin, and 50 μM pyridoxal-5-phosphate to the cell lysis buffer. Since the baseline value of ODC activity varied from day to day in control cells, results of each set of experiments are expressed as an ODC activity ratio (the mean ratio of the enzyme activities of exposed cultures to those of corresponding controls).

RESULTS AND DISCUSSION:

Cultures exposed to continuous wave (CW) microwaves (SAR of 3 mW/g) for periods of 2 to 24 h showed no significant alterations in ODC activities Figure 1.I. In contrast, cultures exposed to the same conditions, but with 60 Hz amplitude modulation at 23%, displayed a transient doubling of ODC activity (1.9 $\pm$ 0.3) relative to matched controls, which peaked at approximately 8 h of exposure. Despite continued exposures ODC activities declined from this peak, reaching control values by 16 to 24 h. Eight hour exposures using an SAR of 0.6 mW/g yielded similar ODC enhancement.

The two-fold enhancement of ODC activity in the presence of the 60 Hz AM field was highly significant (p < 10^{-4}). Further, the increased enzyme activity was not due to heating of the culture medium. Both CW and 60 Hz AM fields were at the same SAR, yet only the AM field produced increased ODC activity, and changes in temperature of the culture medium during microwave exposure were found to be less than 0.1° at an SAR of 3 mW/g.

Figure 1.I: Ornithine decarboxylase activity ratios for L929 cultures exposed to either continuous wave or 60 Hz AM, 915 MHz microwaves.

Since the 60 Hz AM, but not the CW microwave field produced enhanced ODC activity, effects of additional AM frequencies were studied. A time course of 2 to 24 h exposure was conducted using a 16 Hz AM field, and 8 h exposures were conducted for 6 and 600 Hz AM fields. In all cases amplitude modulation was 23% and SAR was
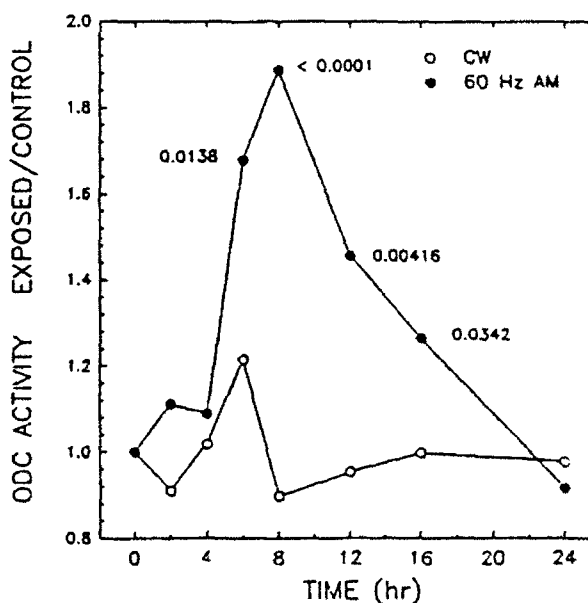
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maintained at 3 mW/g. As shown in Figure 2.I, there was no significant difference in ODC activity of exposed cultures relative to matched controls for any of these conditions.

These data confirmed the basic observation of Byus et al. that AM microwave fields can produce transient enhancement of ODC activity. In some respects, however, the data from the two projects are discordant. Byus et al. (2) did not observe elevated ODC activity following exposure of cultures to 60 Hz AM 450 MHz microwaves. This may be explained by the fact that they exposed cells for only 1 h to the microwave field, and collected cells at a maximum of 4 h after onset of exposure. We observed no definite enhancement of ODC activity in L929 cultures after 4 h of 60 Hz AM, so it is probable that with longer exposure Byus et al. would have seen a 60 Hz AM stimulation. Our failure to observe their 16 Hz AM-induced stimulation of ODC activity may reflect differences in the degree of percent amplitude modulation (23% vs 85%), or other differences in exposure conditions.

Figure 2.I: Ornithine decarboxylase ratios for L929 cultures exposed to continuous wave, or 6, 16 or 600 Hz AM, 915 MHz microwaves.

Microwave-exposed cultures, thus, demonstrated an enhancement of ODC activity, but only when there was 60 Hz amplitude modulation of the microwave field. This result is in agreement with the requirement of amplitude modulation for the production of microwave field-induced efflux of Ca^{++} from brain tissue (5). Since ODC enhancement required the presence of the 60 Hz amplitude modulation frequency, the response should be seen primarily as an ELF effect, rather than a microwave-specific effect. In fact, the idea of an ELF-induced ODC response is substantiated by the observation of Byus et al. that ODC activity in cultured cells exposed to a sinusoidally varied, 60 Hz electric field (10 mV/cm) increased by factors of 0.4-5 times (3). In order to confirm such ELF effects, we undertook verification of a 60 Hz response in L929 cells.

Cells exposed to a 10 μT, 60 Hz magnetic field produced by vertically oriented Helmholtz coils displayed a transient, approximately two-fold (2.10 $\pm$ 0.32) increase in ODC activity in a manner similar to that seen for cells subjected to 60 Hz AM microwaves (Fig 3.I). The time course for this enhancement was shorter than that observed for the microwave response, with ODC activity peaking at approximately 4 h of exposure and returning to control values by 8 h. Increasing the magnetic field to 100 μT also yielded an approx. doubling of ODC activity, while a decrease in field amplitude to 1 μT produced no significant enhancement relative to matched controls.
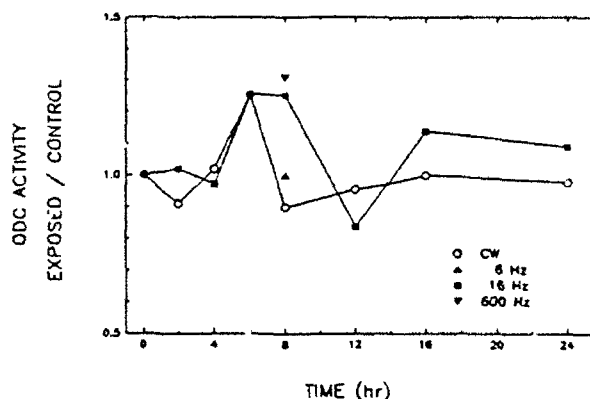
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The L929 response to 60 Hz magnetic fields was confirmed in the Daudi human lymphoma cell line. For these cells ODC activity peaked after 6 h of exposure to the 10 μT field (2.23 $\pm$ 0.31). The 2 h difference noted between L929 and Daudi cells in time to reach maximal ODC activity in the field was similar to differences reported by Byus *et al.* (3) for the field-induced ODC response in different mammalian cell types.
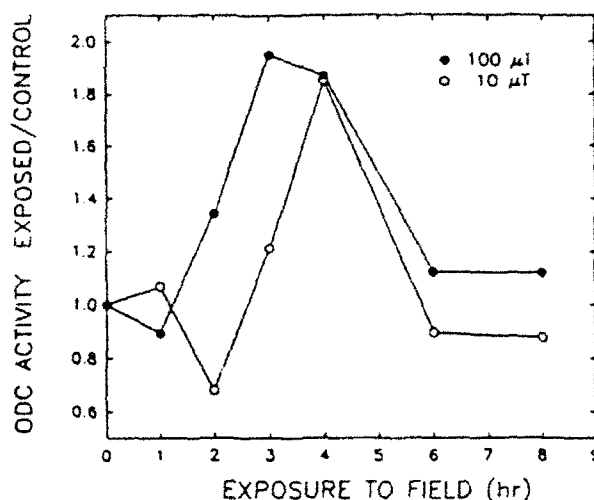
Figure 3.1: Ornithine decarboxylase activity ratios for L929 cultures exposed to 60 Hz, sinusoidal magnetic fields of 10 and 100 μT.

Considerable similarities were found in the ODC response of L929 cells to both 60 Hz AM microwave and 60 Hz magnetic fields. In either case a transient, approximate doubling of ODC activity was produced. The fact that a 60 Hz amplitude modulation was required to produce an ODC response from the microwave signal, and that a 60 Hz magnetic field was also effective, indicates that the cells must be producing some sort of demodulation of the AM microwave field, and responding to the resultant ELF field. The mechanism of such demodulation is not clear. None the less, ODC was shown to serve as a reliable marker to monitor cellular response to both AM microwave and ELF fields. Additionally, since alterations in ODC activity are associated with cell transformation (6), and since elevated ODC activity has been demonstrated to enhance transformation of cells by an activated c-H-*ras* oncogene (7), ODC is also of potential direct interest for the question of adverse health effects resulting from EMF exposure.

REFERENCES Ic:

1.   Heby, O., and L. Persson. 1990. Molecular genetics of polyamine synthesis in eukaryotic cells. Trends in Biochemical Sciences 15: 153-158.

2.   Byus, C. V., K. Kartun, S. Pieper, and W. R. Adey. 1988. Increased ornithine decarboxylase activity in cultured cells exposed to low energy modulated microwave fields and phorbol ester tumor promoters. Cancer Research 48: 4222-4226.

3.   Byus, C. V., S. E. Pieper, and W. R. Adey. 1987. The effects of low-energy 60-Hz environmental fields upon the growth-related enzyme ornithine decarboxylase. Carcinogenesis (Lond.) 8: 1385-1389.

4.   Seeley, J.E. and A.E. Pegg. 1983. Ornithine Decarboxylase (Mouse Kidney). Methods in Enzymology, 94:158-161.

5.   Adey, W.R. 1980. Frequency and power windowing in tissue interactions with weak electromagnetic fields. Proc. IEEE, 68:119-125.

6.   *Russell, D.H. 1985. Drug Metabolism Reviews, 16:1-88.*

7.   *Hibshoosh, H. M. Johnson and I.B. Weinstein. 1991. Effects of over expression of ornithine decarboxylase (ODC) on growth control and oncogene-induced cell transformation. Oncogene, 6:739-744.*

d. Requirement of Field Coherence for Bioresponses

Results from cell culture studies have documented alterations in cell metabolism after exposures to extremely low frequency electromagnetic fields (EMFs) (1). Many such reports of EMF effects on biological systems have been obtained with applied extremely low frequency (ELF) fields as weak as 1 μT magnetic intensity with associated fields below 1 μV/cm. The field strengths are well below the thermal noise fields generated by the random motion of ions in the vicinity of the cell (2,3). This *signal-to-noise* mystery lies at the core of the problem of unraveling the mechanisms by which cells detect and respond to EMFs.

In an attempt to resolve this conundrum, we proposed the possibility that the cell's signal transduction mechanism might demand a certain degree of coherence in the applied fields before it would respond to them. In this way the thermal field would be ignored by the cell. This concept was explored experimentally by considering whether, during exposure, a time varying EMF must maintain coherence over some minimum interval to elicit a cellular response. Coherence in the sense used here refers to the maintenance of a "steady-state" field, one in which the field parameters (frequency, phase, wave form, direction and amplitude) are sensibly constant. The organizing hypothesis is that living cells respond only to impressed EMF that are "stead-state" for some minimum coherence time. If any of these parameters is caused to vary on a time scale shorter than this, the field induces no biochemical response in the cells. The idea of a stead-state character to the field is a "coarse-grained" one in that variations on a time scale of less than 0.1 second or so are ignored.

The following three sections document the experimental protocols used to test the concept of a field coherence requirement, and provide data that demonstrate the validity of the hypothesis.

REFERENCES Id:

1.   Byus, G.V., S.E. Peiper and W.R. Adey. 1987. *Carcinogenesis*. 8: 1385-1389.

2.   Weaver, J.C. and R.D. Astumian. 1990. *The response of living cells to very weak electric fields: the thermal noise limit. Science*, 47: 459-462.

3.   Adair, R.K. 1991. *Constraints on biological effects of weak extremely-low-frequency electromagnetic fields. Phys. Rev.*, A43: 1039-1048.

i. The Effect of Microwaves on Ornithine Decarboxylase Activity: The Role of Coherence Time

SUMMARY

Eight-hour exposure to a 55-, 60- or 65-Hz amplitude modulated 915 MHz microwave field approximately doubles the specific activity of ornithine decarboxylase (ODC) in L929 murine (mouse) cells. Partial incoherence was introduced into the amplitude modulation signal by shifting the modulation frequency between 55- to 65-Hz at intervals of $\tau_{coh} - \delta\tau$, where τ_{coh} is a predetermined time interval and $\delta\tau << \tau_{coh}$ varies randomly from one frequency shift to the next. To obtain the full ODC enhancement, it was found that the coherence of the amplitude modulation signal must be maintained for a minimum of about 10s. For $\tau_{coh} = 5.0s$ a partial enhancement is elicited, and at 1.0s there is no bio-response.

INTRODUCTION:

Concern over adverse health effects resulting from exposure to electromagnetic fields (EM) has generated an increasing effort to determine how fields interact with biological systems. Results from cell culture studies have documented alterations in cell metabolism after exposures to amplitude modulated microwave (1) and extremely low frequency fields (2). Such data make it clear that EM fields interact with cells and affect their metabolism, but, the mechanisms of the interaction are not understood.

Given the limited ability of an electric field to penetrate the cell membrane, it is a reasonable assumption that the most likely place for a field to interact with the cell is at protein receptors exposed on the external membrane surface, with fields affecting the cell's physiology via signal transduction systems. Our group proposed the possibility that the cell's signal transduction mechanism might demand a certain degree of coherence in the applied fields before it would respond to them. This concept was explored experimentally by considering whether, during exposure, the frequency of amplitude modulation of a microwave field must maintain coherence over some minimum interval to elicit a cellular response. The coherence time is loosely defined as the time interval over which one can reasonably predict the period, phase, wave form, direction, and amplitude of the field. The biological endpoint selected for this purpose was the AM microwave-induced enhancement of specific activity for the enzyme ornithine decarboxylase (ODC) in murine L929 fibroblasts.

MATERIALS AND METHODS:

Logarithmically growing cultures of murine L929 cells, maintained in Eagle's minimum essential culture medium with 5% fetal bovine serum, were plated 24 h prior to magnetic field exposure. To avoid serum stimulation of ODC activity, the culture medium was not changed before experiments were begun. For each experiment four 25 cm^2 flasks of logarithmically growing cells were microwave-exposed using a Crawford Cell system maintained at 37° in an incubator chamber. 915 MHz microwaves, amplitude modulated (23%) by sine waves at 55, 60 or 65 Hz were used. The specific absorption rate (SAR) of 3 mW/g produced temperature changes of less than 0.1° in the culture medium. Four matched control flasks were maintained outside the Crawford Cell in the same incubator chamber.

A series of exposures was conducted in which the amplitude modulation frequency was maintained constant at 55, 60 or 65 Hz. Additional series were conducted in which all microwave conditions were maintained constant except for a switching of the amplitude modulation frequency from 55 to 65 Hz at intervals ranging from 0.1 to 50.0 s. This switching was done by a computer program which interfaced with a function generator to determine the modulation frequency and also the time interval for which a given frequency was maintained. At user-selected intervals (henceforth termed coherence times, or τ_{coh}) the AM frequency was alternately shifted from 55 Hz to 65 Hz (see Figure 4.I). The phase of successive intervals was randomized by inserting a small uncertainty in τ_{coh}. Thus the time between frequency shifts was actually τ_{coh} - δt where $\delta t \ll \tau_{coh}$ and is a random time which varied between 0 and 0.05 s.
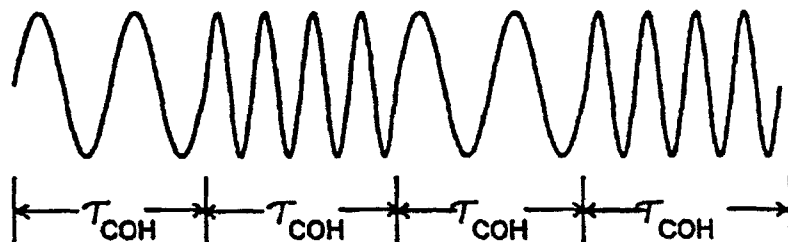

Figure 4.I: A plot demonstrating the partially coherent wave form created by shifting amplitude modulation frequencies from 55- to 65- Hz at intervals of time, $\tau_{coh} \pm \delta\tau$, where $\delta\tau$ is a random number ($\ll \tau_{coh}$) varying between 0 and .05 s.

Following exposure cell pellets were collected and frozen for subsequent analysis of ornithine decarboxylase activity. Ornithine decarboxylase activity was determined as reported (1). Results, described below, are expressed as a ratio (ODC activity ratio) of the ornithine decarboxylase specific activity of an exposed culture to that of its matched control. Several experiments were conducted for each exposure condition (i.e. for each modulation frequency or τ_{coh}), with results given as the mean activity ratio $\pm$ SD.

RESULTS AND DISCUSSION:

An approximate doubling (1.87 $\pm$ 0.34 relative to matched controls) of ornithine decarboxylase activity was obtained for L929 cultures exposed to the 60 Hz AM microwave field for 8 h. However, no ornithine decarboxylase enhancement was observed for cultures exposed to the unmodulated 915 MHz microwave field. The extremely low frequency of the amplitude modulation, thus, was the critical factor in eliciting cellular response. In order to examine the importance of the coherence time, τ_{coh}, for the microwave response, ornithine decarboxylase activities of cultures exposed to microwave fields amplitude modulated at 55 or 65 Hz were assessed. Results, 1.88 $\pm$ 0.50 and 2.07 $\pm$ 0.40, respectively, were statistically indistinguishable from those obtained with 60 Hz.

Results of the coherence time studies are tabulated in Table 1.I displayed graphically in Figure 5.I. When the modulation frequency was shifted at τ_{coh} of 0.1 or 1.0 sec no significant enhancement of ornithine decarboxylase activity over control levels was observed. However a τ_{coh} of 10 s yielded an enhancement of ornithine decarboxylase activity equivalent to that obtained with constant AM modulated frequencies of 55, 60 or 65 Hz. Increasing the τ_{coh} five-fold to 50 s produced an enhancement equivalent to that obtained at 10 s. Thus, approximately two-fold increases in activity were obtained with values of τ_{coh} of 10.0 s or greater. A 5 s coherence time produced a level of enhancement that was intermediate between control values and those obtained with τ_{coh} of 10 s or longer.

For the modulated microwave data the R_{ODC} activity ratio, [ODC], was fit to the function,

$$R_{ODC} = 1 + 1.23 \left(1 - e^{-\frac{\tau_{coh}}{\tau_{cell}}} \right).$$

with best fit value of τ_{cell} = 5.3 $\pm$ 2.2 s. Thus, there appears to be some fundamental (but unexplained) time constant, τ_{cell}, associated with the cell signal transduction mechanism. For the cell to respond to an AM microwave signal it is necessary for the amplitude modulation frequency to maintain coherence for a minimum time interval greater than about several seconds, with full response requiring an interval greater than about 10.0 s.

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TABLE 1.I: ODC activity induced by microwaves, listed as a function of coherence time.

COHERENCE TIME (τ_{coh})	ODC ACTIVITY RATIO (exposed/control)
0.1	1.02 ± 0.26
1.0	1.02 ± 0.21
5.0	1.52 ± 0.15
10.0	2.13 ± 0.38
50.0	2.00 ± 0.26

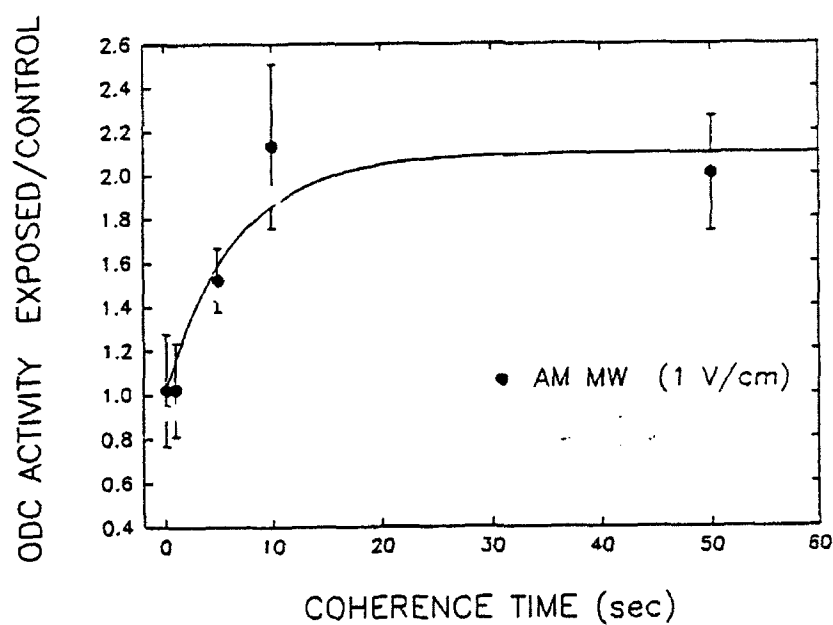

FIGURE 5.I: Ornithine decarboxylase activities resulting from variation of the modulation frequency at specific τ_{coh}.

Enhancement of ornithine decarboxylase activity by exposure to microwaves requires that the microwave carrier be amplitude modulated. It is, thus, the extremely low frequency of the modulating signal which is critical to producing the ornithine decarboxylase response. The mechanism by which the AM signal affects the cell is not known. However, it does appear that the cell somehow demodulates the microwave signal and that the demodulated ELF stimulus is what affects the cell function.

REFERENCES Idi:

1. *Byus, C. V., K. Kartun, S. Pieper, and W. R. Adey. 1988. Increased ornithine decarboxylase activity in cultured cells exposed to low energy modulated microwave fields and phorbol ester tumor promoters. Cancer Research 48: 4222-4226.*

2. *Litovitz, T.A., D. Krause and J.M. Mullins. 1991. Effect of coherence time of the applied magnetic field on ornithine decarboxylase activity. Biochem. Biophys. Res. Comm., 178:862-865.*

ii. Effect of Low Frequency Magnetic Fields on Ornithine Decarboxylase Activity: Role of Coherence Time

SUMMARY

Four-hour exposure to a 55- or 65-Hz magnetic field approximately doubles the specific activity of ornithine decarboxylase (ODC) in L929 murine (mouse) cells. Partial incoherence was introduced into the applied field by shifting the frequency between 55- to 65-Hz at intervals of τ_{coh} - $\delta\tau$ where τ_{coh} is a predetermined time interval and $\delta\tau$ << τ_{coh} varies randomly from one frequency shift to the next. To obtain the full ODC enhancement, it was found that the coherence of the impressed signal must be maintained for a minimum of about 10s. For τ_{coh} = 5.0s a partial enhancement is elicited, and at 1.0s there is no bio-response.

INTRODUCTION:

In the previous section, it was demonstrated that the amplitude modulation frequency of a microwave signal must have a minimum coherence time if the field is to produce an enhancement of ornithine decarboxylase activity in L929 fibroblasts. Further investigation has revealed a remarkably similar coherence time pheno: .enon for enhancement of ornithine decarboxylase activity by extremely low frequency (ELF) magnetic fields. Magnetic fields with frequencies of 55, 60 or 65 Hz approximately doubled ornithine decarboxylase activity after 4 h. Switching frequencies from 55 to 65 Hz (during 4 h exposure) at coherence times (τ_{coh}) of 1.0 s or less abolished enhancement, while times of 10 seconds or longer provided full enhancement.

METHODS:

Logarithmically growing cultures of murine L929 cells were maintained as described in the preceding section. ELF exposures were conducted using incubator-housed Helmholtz coils to produce a sinusoidal, 55, 60 or 65 Hz horizontal magnetic field of 10 μT. Four 25 cm^2 flasks of cells were used for each exposure, and to serve as controls four identical flasks were placed in an incubator chamber adjacent to that housing the Helmholtz coils. The τ_{coh} were determined essentially as reported for AM microwave fields (see Section Idi). A computer was interfaced with a function generator to alter the ELF frequency from 55 to 65 Hz at user-selected intervals. A random time ($\delta\tau$), varying from 0 to 0.05 s, was subtracted from each interval to assure randomization of phase upon switching.

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At the end of exposure cells were harvested by gentle scraping, washed with phosphate buffered saline and stored as frozen pellets. Ornithine decarboxylase activities were assayed as reported (1). Results of each set of experiments are expressed as the mean ratio of the enzyme activities of exposed cultures to those of the corresponding controls ($\pm$ SEM).

RESULTS AND DISCUSSION

Cultures were subjected, in a series of exposures, to 60 Hz magnetic fields of 10 μT for times ranging from 1 to 8 hr. The enhancement of ODC activity was measured in terms of the ratio of exposed/control activity. Maximal enhancement of ODC activity (2.04 $\pm$ 0.21) was produced by 4 hr exposure. The induced electric field was approximately .04 μV/cm. Comparable enhancements of ODC activity (1.79 $\pm$ 0.20, 2.10 $\pm$ 0.35) were obtained with frequencies of either 55 or 65 Hz. Using 4 hour exposures, 10 μT fields, and frequencies shifting alternately between 55- and 65-Hz, the coherence times were varied from 0.1 to 50.0 seconds.

TABLE 2.I: ODC activity induced by 60 Hz EM fields as a function of coherence time.

Coherence Time τ_{coh} (seconds)	ODC Activity exposed/control values
0.1	0.90 $\pm$ 0.11
1.0	0.93 $\pm$ 0.17
5.0	1.45 $\pm$ 0.10
10.0	1.90 $\pm$ 0.14
50.0	2.08 $\pm$ 0.24

The results are listed in Table 2.I and are plotted in Figure 6.I. They show that application of fields for four hours, but with coherence times of 10 or 50 s, did produce enhancements in ODC activities. The amount of enhancement was (within experimental accuracy) the same as that observed after EM exposures which were coherent for the full four hours of exposure. In contrast, for a coherence time of 0.1 or 1.0 s no enhancement of ODC activity was observed. A 5 s coherence time produced a level of enhancement (1.54 $\pm$ 0.06) that was intermediate between control values and those obtained with τ_{coh} of 10 s or longer.
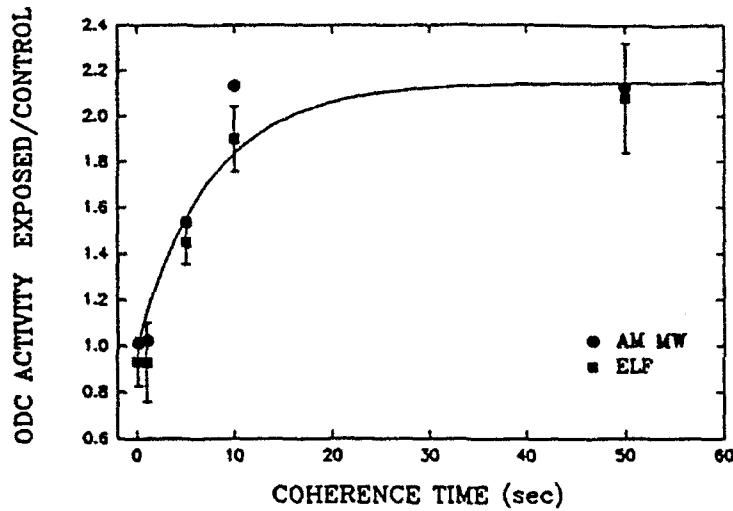
FIGURE 6.1: ODC activity ratios for L929 cultures exposed to 55 and 65 Hz magnetic fields at specific τ_{coh}. Results are also shown for the corresponding microwave studies (Section Idi).

For the ELF data the ODC activity ratio, R_{ODC}, was fit to the same function as in the AM microwave coherence study (preceding section). That is,

$$R_{ODC} = 1 + A\left(1 - e^{-\frac{\tau_{coh}}{\tau_{cell}}}\right) .$$

where A is a constant associated with the magnitude of the enhancement and τ_{cell} is a time constant apparently associated with the cell detection mechanism. The fit parameters are listed in Table 2.I.

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Parameter	Microwave Exposed	ELF Exposed	Combined ELF and Microwave
A	1.08 ± .15	1.11 ± .15	1.1 ± 1.6
τ_{cell} (sec)	5.3 ± 2.2	8.2 ± 2.9	6.6 ± 1.6
Electric field (μV/cm)	≈ 5 x 10^5	≈ .04	—

In summary it has been demonstrated that 60 Hz amplitude modulated 915 MHz microwave fields, and 60 Hz ELF fields, both produce a transient increase in the ornithine decarboxylase activity of L929 cells. The maximum value of this increase is in both experiments a factor of about 2. Data presented here show that the ELF and amplitude modulated microwave effects share an additional, and striking, similarity in the requirement for maintenance of coherence over some minimum time interval. In each case the use of a τ_{coh} of 0.1 or 1.0 s resulted in no ornithine decarboxylase enhancement, 5.0 s produced a value intermediate between control and the maximum electromagnetic field-induced values, and 10.0 s or longer yielded full enhancement. It can be seen by comparing the microwave and ELF data that the variation of R_{ODC} with τ_{coh} is quite similar.

In the above study (Section Idi) on the role of coherence in AM microwave effects on ornithine decarboxylase activity it also was possible to fit the results to Eq.(1). The fit parameters are listed in Table 3.I. It can be seen that there is good agreement between the fit parameters for the two experiments. The values of τ_{cell} for the modulated microwave and ELF are the same to within the uncertainty of the fit. This is further indication that τ_{cell} is truly some fundamental time constant of the cell.

Also tabulated in Table 3.I are the values of the electric fields used in each experiment. It is seen that, although the electric field strengths differ by a factor of 100,000, the values of τ_{cell} are the same.

There are those who (based on signal-to-noise considerations) would say that it is impossible for electromagnetic fields as small as 1 μV/cm to cause any biological effects (1). They would agree that electromagnetic fields as large as 10^5 μV/cm could and probably do have an effect. The agreement found here between the μV/cm ELF data and the V/cm microwave data offers further proof that observed μV/cm ELF effects are real and that the requirement of a minimum coherence time will have to be included in any future considerations of the bioeffects of electromagnetic fields.

REFERENCES Idii:

1.   Litovitz, T.A., D. Krause and J.M. Mullins. 1991. *Effect of coherence time of the applied magnetic field on ornithine decarboxylase activity.* Biochem. Biophys. Res. Comm., 178:862-865.

2.   Adair, R.K. 1991. Phys. Rev. A43:1039-1048.

e.   Effect of Superimposed EM Noise on the Ability of Coherent EM Fields to Modify ODC Activity in Cultures of Murine L929 and Human Lymphoma Cells

SUMMARY

L929 murine fibroblasts display a doubling of ornithine decarboxylase activity following 4-h exposure to a 60-Hz, sinusoidal magnetic field of 10 µT. Simultaneous application of a spatially coherent electromagnetic noise field of comparable amplitude eliminated enhancement of ornithine decarboxylase activity by the stimulating field. Lowering of the amplitude of the applied noise field restored field-induced enhancement of enzyme activity. These results are discussed in terms of the thermal noise limit for weak field detection.

INTRODUCTION:

Members of our group have examined the question of the thermal noise limit for detection of weak electromagnetic fields (EMF) by cells. They reasoned that the spatially random nature of a thermal noise field is fundamentally different from that of spatially coherent, applied electromagnetic fields, and that this difference could account for the ability of cells to detect weak applied fields without thermal noise interference. Details of this argument are presented in elsewhere in this report. According to this hypothesis, biological response to a stimulating field should be eliminated if a spatially coherent noise field of comparable amplitude is simultaneously applied.

To examine the effects of spatially coherent noise on cellular response to EMF we have assayed enhancement of ornithine decarboxylase (ODC) activity. Logarithmically growing cultures of L929 cells display a doubling in ODC activity after exposure for 4 hours to a 60-Hz, 10-µT magnetic field (1). ODC response was assessed under conditions in which this stimulating field was applied simultaneously with electromagnetic noise fields of amplitudes equal to, or less than, that of the stimulating field.

METHODS:

Cultures of L929 cells were maintained as reported (1). Daudi cells were grown in RPMI 1640 medium, supplemented with 20% fetal bovine serum. Experiments used three 75 cm² flasks of logarithmically growing cells each for control, exposed, and exposed + noise conditions. Stimulating fields (60-Hz, sine wave, average amplitude

10-μT, induced electric field of 0.04 μV/cm) were applied simultaneously with random noise fields (spectral range 30 to 90 Hz; rms amplitude, 0.5 to 10 μT) via vertically oriented Helmholtz coils.

At the end of exposure cells were harvested by gentle scraping, washed with phosphate buffered saline and stored as frozen pellets. Ornithine decarboxylase activities were assayed by the procedure of Seely and Pegg (4), modified by addition of 0.2% Nonidet P-40, 50 μg/ml leupeptin, and 50 μM pyridoxal-5-phosphate to the cell lysis buffer. The results are expressed as either a ratio of enzyme activities of exposed cultures to those of matched controls, or, as the percent of ODC enhancement measured for exposed + noise conditions, relative to that of matched exposed cultures. Since this work was designed to assess affects of noise on ODC enhancement, only those experiments in which the exposed cultures displayed an ODC activity ratio of 1.7 or higher were analyzed.

RESULTS AND DISCUSSION:

Cultures of L929 cells exposed to the 60-Hz stimulating field alone displayed an approximately two-fold enhancement of ODC activity (2.10 ± 0.32 times control values). Simultaneous application of the stimulating field and a random noise field, however, altered this response (Figure 7.I). ODC enhancement was eliminated when the stimulating field was combined with a random noise field of 10 μT rms amplitude, but as the amplitude of the noise field was diminished to 2 and 0.5 μT, ODC enhancement was restored. ODC enhancement in response to the stimulating field was, thus, dependent upon the amplitude of the applied noise field.

The ability of spatially coherent noise to interfere with ODC enhancement was examined also for Daudi human lymphoma cells. Preliminary data show that, for this cell line, a maximal enhancement (2.23 ± 0.31) of ODC activity occurred after a 6-h exposure to the stimulating field. Addition of a 10-μT noise field to the stimulating field eliminated the enhancement of ODC activity.

A spatially coherent electromagnetic noise field, with rms amplitude approximately equal to that of a simultaneously applied stimulating field, thus, inhibited field-induced enhancements of ODC activity in L929 and Daudi cells. The extent of this inhibition was dependent upon the amplitude of the noise field. These data are consistent with the inhibitory effects of spatially coherent noise on field-induced morphological abnormalities in chick embryos (Section Ig, this report). Such similarity in results from work with whole embryos and with cultured cells suggests that the inhibition of field-induced effects by spatially coherent noise represents a general biological response.

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Enhancements of ODC activity thus are induced by $\mu V/cm$ stimulating fields in the presence of random thermal noise fields, but are eliminated by spatially coherent noise fields whose amplitudes are two to three orders of magnitude lower than those of the thermal noise (2). It is not surprising that cells might ignore the presence of thermal noise fields. Since cells experience the constant presence of random EMF produced by

FIGURE 7,I: Ornithine decarboxylase responses of L929 cells exposed simultaneously to a 60 Hz, 10 μT stimulating field and temporally incoherent noise fields.

thermal noise, they surely have evolved in ways that render such stimulation inconsequential. The dilemma to be resolved, then, is the question of why applied EMF far weaker than those originating from thermal noise are of consequence in altering cellular response. The concept of spatial coherence elaborated, in Section Id of this report, provides a hypothesis that accounts for this.

It is a reasonable conjecture that many, if not all, effects of EMF on cells occur through interaction of the field with membrane receptor proteins. If this is so, then the concept of temporal coherence previously reported (1), and the requirement for spatial coherence demonstrated here, should provide clues as to how fields and receptors may actually interact. In fact, the temporal coherence times we have noted are similar to the times required for aggregation of ligand-bound tyrosine kinase receptors (3), and the idea of spatial coherence fits with the general concept of receptor cooperativity (4).

REFERENCES Ie:

1.   Litovitz, T.A., Krause, D., and Mullins, J.M. 1991. Biochem. Biophys. Res. Comm. 178:862-865.
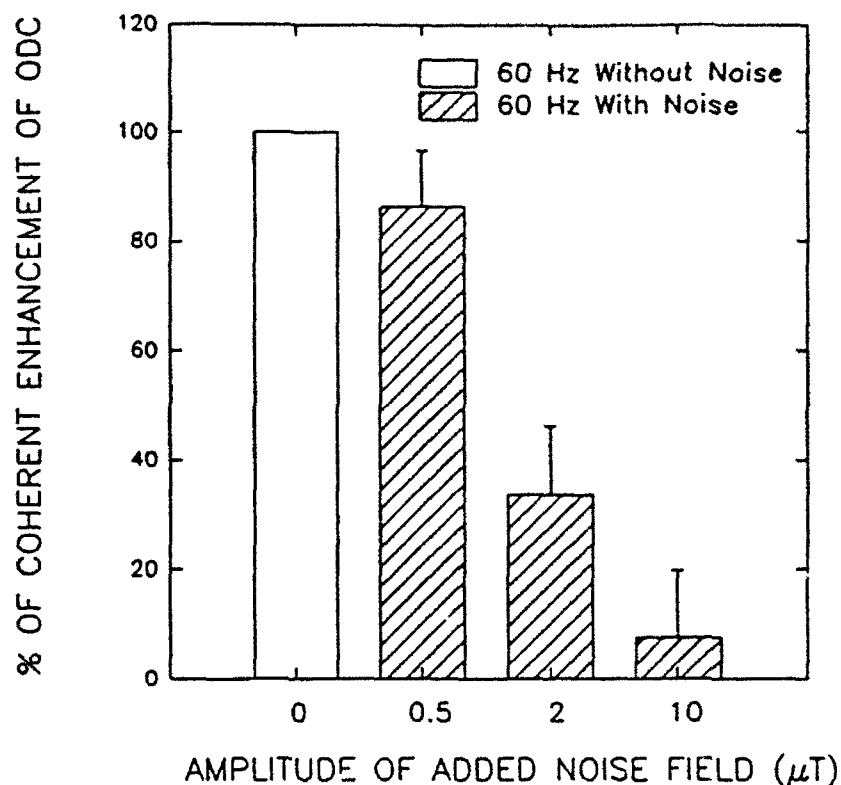
2.   Adair, R.K. 1991. Phys. Rev. A43:1039-1048.

3.   Hollenberg, M.D. 1991. _FASEB J._ 5:178-186.

4.   Morgan, N.G. _Cell Signaling_. Guilford (1989).

f.   Delineation of Electric and Magnetic Field Effects of Extremely Low Frequency Electromagnetic Exposure on Transcription in HL-60 Cells

SUMMARY

Exposure of HL-60 human leukemia cells to a 60 Hz magnetic field produced a transient enhancement of incorporation of labeled uridine into total cellular RNA. The field-induced transcriptional effect was determined to correspond to the magnitude of the electric, rather than the magnetic, field through experiments in which cultures were simultaneously exposed, in the same dish, to the same magnetic field, but different induced electric fields. The RNA species affected was the 45 S pre-ribosomal RNA. Pulse-chase studies showed that processing of the 45 S RNA into 28 S and 18 S rRNAs was also accelerated.

INTRODUCTION:

Among the most intriguing reports involving biological response to extremely low frequency (ELF) electromagnetic fields are those which demonstrate changes in the rate of transcription, or in the steady state levels of, RNA. In some instances dramatic increases in the steady state levels of particular mRNA species have been reported after brief exposures to ELF fields (1). This work, and our efforts to duplicate such results, will be discussed in the following section. Other reports have dealt with more general changes in transcription by monitoring the rate of uptake of ^{3}H-uridine into general RNA populations (2). In such work there have been reported field-induced increases in the rate at which labeled uridine has incorporated into RNAs.

We were intrigued by the possibility of altering transcription of RNAs by exposure to ELF fields. Additionally, in none of the previous work was it possible to discern whether the magnetic field, the electric field, or some combination of the two components was responsible for a change in either RNA synthesis or steady state levels. Such information would be extremely useful in analyzing electromagnetic field effects, and in devising ways by which to inhibit them.

In the work described below we employed a solenoid coil to expose cultures of the human leukemia cell line HL-60 to 60 Hz magnetic fields in the 1 - 10 Gauss range. The magnetic field, which was oriented perpendicularly to the growth surface of the cells, induced an electric field in the culture medium. The induced electric field was 0 at the center of each culture dish, but increased radially towards the edge of the dish. By plating cells in dishes with concentric annular chambers, it was possible to expose 2

populations of cells, simultaneously, to the same magnetic field, but different average electric fields. Results of this work show that it is the induced electric field which relates to changes in transcription of RNA, and that for the 45 S rRNA species affected, both rate of transcription and rate of degradative processing seem to be altered.

Portions of this work have already been published (3).

METHODS:

Cell Culture: HL-60, human leukemia cells, were maintained in logarithmic growth in Eagle's minimum essential medium supplemented with 10% fetal bovine serum. One hour prior to exposure, cells were inoculated into 60mm culture dishes at a density of 1×10^6 cells/ml (cultures for overnight exposure were inoculated at 0.5×10^6 cells/ml). Cells were exposed in either standard 60 mm culture dishes, in organ culture dishes which provided two concentric annular compartments (average radii of 0.90 and 2.03 cm), or dishes machined from lucite, which had two concentric annular chambers (average radii of 0.87 and 2.49 cm).

ELF Exposure: Exposure to magnetic fields was accomplished with a solenoid chamber. The chamber was supplied with a thermostated water circulation system to maintain the temperature of the cultures at $37° \pm 0.1°$ during exposure. A water-jacketed, 37°, cell culture incubator was used to house the solenoid system. Matched control dishes were housed in the same incubator chamber, at a distance from the solenoid where no measurable fields from the solenoid system could be detected. More thorough descriptions of the solenoid system are provided in the Electrical Engineering sections of this report. The magnetic field, which was oriented vertically with respect to the growth surfaces of the culture dishes, was uniform across the diameter of the dishes. Magnetic fields ranged from 1 to 10 Gauss. The induced electric field in the culture medium was 0 mV/m at the center of the dish, and increased radially toward the dish periphery. This system provided a means of exposing cells in two concentric annular chambers to the same magnetic field, but different average electric fields.

Radiolabeling: Labeling of newly transcribed RNA was accomplished by addition of ^{3}H-uridine (20 μCi/ml) to the culture medium. For continuous labeling ^{3}H-uridine was added at the onset of exposure. Pulse labeling was accomplished by addition of the ^{3}H-uridine for the last 15 m of electromagnetic field exposure. After labeling cells were harvested by centrifugation, lysed and precipitated with trichloroacetic acid. The precipitated material, containing cellular RNA, was collected on filters and quantified by liquid scintillation counting.

Pulse-chase experiments were conducted by pulse labeling cultures with ^{3}H-uridine for the last 15 m of exposure to the magnetic field, removal of the labeling medium, washing of the cells and addition of new culture medium to which a 100X excess of unlabeled uridine had been added. Cultures were then returned to the incubator for chase

periods ranging from 15 to 180 m. At the end of the chase period RNA was isolated and electrophoresed. After electrophoresis the gel was cut into slices and the labeling of each slice was determined by liquid scintillation counting.

RESULTS AND DISCUSSION:

Field-Induced Changes in Rate of Transcription:

Changes in the rate of incorporation of ^{3}H-uridine into total cellular RNA were revealed by pulse labeling. For the initial work, cells were grown in standard, 60 mm culture dishes. Cultures were, thus, exposed to a uniform magnetic field, but a radially varying induced electric field. Sixty Hertz fields of 10 Gauss produced a 30 to 50% increase in the incorporation of label by 30 m (Fig. 8.I). Enhanced incorporation of labeled uridine was sustained for about 5 h, after which the levels declined to near control levels by 15-20 h. Fractionation of the RNA showed that the increased transcription could largely be accounted for by an acceleration in the transcription of the ribosomal 45 S precursor RNA. By use of oligo-dT columns, attempts were made to determine any general effects of the field on transcription of mRNAs. No specific increase in labeling of this fraction was determined, but the mRNA f~ction obtained was small, and so minor changes in labeling would not have been detected. Subsequent work, reported by Phillips and McChesney (2), documented enhanced labeling of both total and messenger RNA fractions after exposure of a T-lymphoblastoid cell line to pulsed magnetic fields with peak power of 3.5 mT (35 Gauss). Qualitatively, the changes they observed are similar to those reported here, although transcriptional enhancements of more than 3-fold were observed for these larger fields.

These results serve to confirm an effect of electromagnetic fields on transcription of at least one category of RNA. A change in the incorporation of ^{3}H-uridine into RNA during the relatively brief 15 m pulse period would most likely be due to a change in the rate of transcription, although alterations in the rate of RNA degradation might also be of importance.
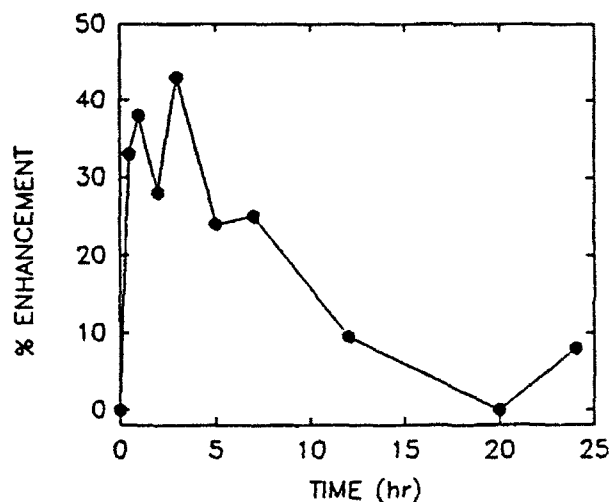

FIGURE 8.I: Enhancement of transcription of total cellular RNA by a 60 Hz, 10 Gauss magnetic field in terms of percent enhancement relative to transcription in matched control cultures.

Delineation of Magnetic vs Electric Field Effects on Transcription:

In order to distinguish between magnetic field and electric field contributions to the transient enhancement of 45 S RNA labeling, exposures were done using dishes with concentric chambers. The results described here were obtained for organ culture dishes which provided a central, circular chamber and a concentric outer chamber. Similar results were obtained for fabricated lucite dishes in which both chambers were of annular form.

Cultures exposed to the same 10 Gauss magnetic field, but to different induced electric fields due to the location of different compartments in the culture dish, displayed different rates of incorporation of ^{3}H-uridine into total RNA. As shown in Figure 9A.I , cells in the outermost well of the organ culture dishes, where the average electric field was 3.40 mV/m, displayed a higher rate of incorporation than did cells in the center well, where the average electric field was 0.34 mV/m. Cells from both compartments displayed similar patterns of uridine incorporation, with an initial increase, followed by a decrease and then a subsequent rise; maximal incorporation of label was, however, approximately 1 h later for cells in the inner chamber.

Differences noted between different culture chambers under 10 Gauss conditions strongly indicated that the field-induced change in transcription corresponded to differences in induced electric fields. To test this idea further, cells in the outer wells of organ culture dishes were pulse labeled under conditions in which the magnetic field was reduced to 1 Gauss. At this magnetic field cells in the outer well experienced an average induced electric field of 0.34 mV/v, the same induced electric field induced in the inner well at 10 Gauss. Figure 9B.I compares the incorporation of ^{3}H-uridine into RNA for conditions which were identical for average induced electric field, but for which there was a 10-fold difference in magnetic field. Incorporation under these conditions is virtually identical, demonstrating convincingly that it is the electric field, and not the magnetic field, which influences transcription.

Continuous Labeling and Processing of the 45 S rRNA Precursor:

We assumed that the field-induced increased incorporation of labeled uridine would manifest itself as a time-dependent increase in the total amount of labeled RNA. To verify this hypothesis, 30 and 60 m exposures to the 10 Gauss field were conducted with ^{3}H-uridine present for the entire exposure period. To our surprise, over a 30-60 m period, there was no difference in total incorporation of label between exposed and control cultures Figure 10.I.

Figure 9A.I: Enhancement of transcription for 2 HL-60 cultures exposed to the same 10 G, 60 Hz magnetic field, but to electric fields of 3.40 (filled circles) and 0.34 (open circles) mV/m.

Since, in the presence of a field, ^{3}H-uridine incorporation was increased by pulse, but not continuous, labeling, we wondered whether processing of the 45 S rRNA precursor might be accelerated by the field. If this were true, then an increased rate of transcription of the 45 S species might be balanced by an increased rate of hydrolytic processing of the 45 S RNA into 28 S, 18 S and 5.8 S rRNAs. This accelerated processing of the 45 S species into mature rRNAs would, most likely, also result in an accelerated rate of degradation of the rRNAs, and so IN the
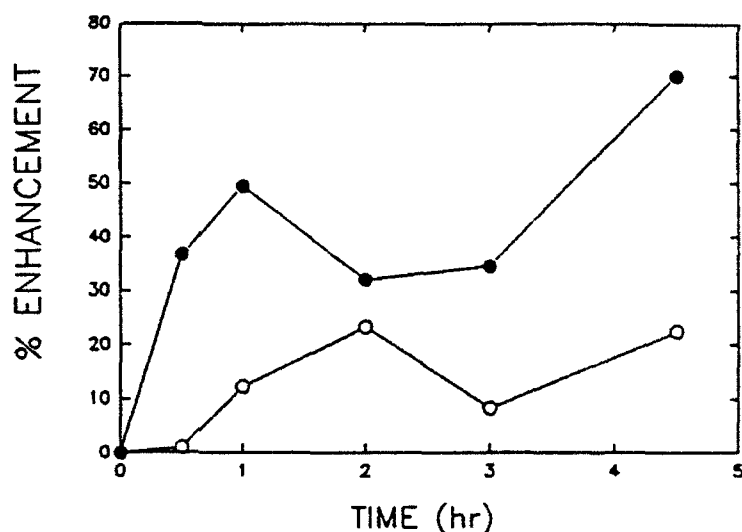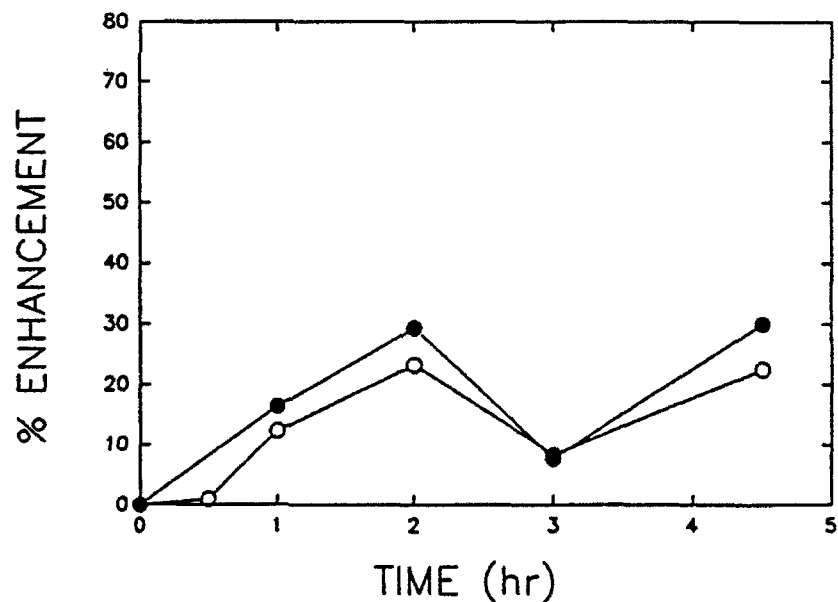

Figure 9B.I: Enhancement of transcription for two HL-60 cultures exposed the same 60 Hz electric field (0.34 mV/m) but to different magnetic fields. ● - 1 Gauss; O - 10 Gauss.

elimination of their ^{3}H-uridine from the cell's total RNA. The processing of the 45 S RNA was tracked, using pulse-chase techniques, gel electrophoresis and scintillation counting, to examine this possibility.

RNA from cultures exposed for 60 m to a 10 Gauss magnetic field, with pulse labeling during the last 15 m of exposure, was analyzed after chase periods of either 15 or 90 m. Results are presented in Figures 11.I and 12.I. Figure 11.I illustrates the processing of the 45 S RNA with time. Comparison of the 15 and 90 m chase curves, for either control or exposed cultures, clearly illustrates the time-dependent diminution of label in the 45 S RNA, and the concomitant increase in that of 28 S and 18 S rRNAs. Exposed cultures, however, show greater amounts of label incorporated into the 45 S RNA after the 15 m chase, and consequently,

Figure 10.I: Results from continuous labeling of HL-60 cells during electromagnetic field exposure. Exposed cells, filled circles; matched control cells, open circles.

greater amounts of label in the two rRNAs after 90 m chase. Figure 12.I analyzes the decline in labeling of the 45 S RNA, and its appearance in the 28 S rRNA, with chase periods ranging from 60 to 180 m. These data indicate a more rapid processing of the 45 S into the 28 S species under conditions of exposure, with this difference occurring primarily between 60 and 90 m of chase.

FIGURE 11.I: Comparison of pulse chase labeling for different RNA fractions. Exposed = closed symbols; control = open symbols.

Taken together, these data demonstrate an enhanced rate of transcription for the 45 S pre-ribosomal RNA species under the influence of an electromagnetic field, and convincingly argue for an effect of the electric, but not the magnetic field component. Enhanced transcription is apparently balanced by an increased hydrolytic processing of 45 S RNA into the mature 28 S and 18 S rRNA species. If the mature species are more rapidly accumulated, then degradation of

these rRNAs would occur also at a more rapid rate, maintaining a
relatively constant level of labeling during the course of exposures up to 60 m duration.
Electric field effects may, thus, be multiple, affecting both rates of synthesis and rates
of degradation of an RNA species. The relative abundance of a given species may
depend upon which of these effects is more greatly influenced.
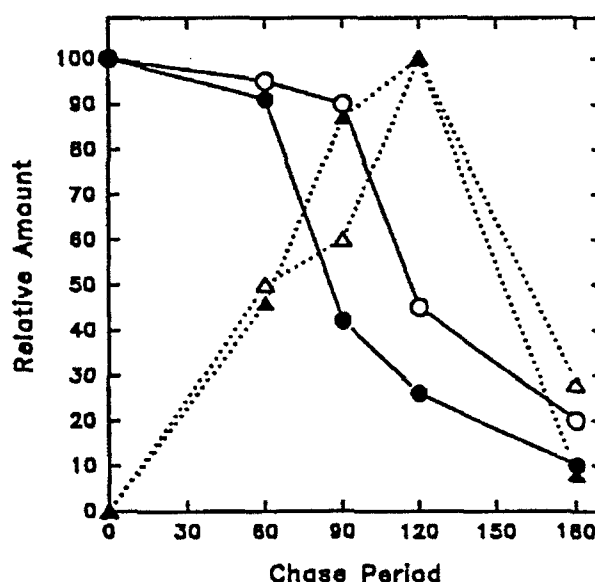
FIGURE 12.I: Pulse chase results comparing levels of 45 S (circles)
and 28 S (triangles) RNAs from exposed (filled symbols) and control
(open symbols) HL-60 cells.

REFERENCES If:

1.   Goodman, R., L. X. Wei, J. C. Xu, and A. Henderson. 1989. Exposure of Human Cells to
      Low-Frequency Electromagnetic Fields Results in Quantitative Changes in Transcripts. Biochimica
      Et Biophysica Acta 1009: 216-220.

2.   Phillips, J. L., and L. McChesney. 1991. Effect of 72 Hz pulsed magnetic field exposure on
      macromolecular synthesis in CCRF-CEM cells. Cancer Biochem. Biophys. 12: 1-7.

3.   Greene, J.J., W. J. Skowronski, J.M. Mullins, R.M. Nardone, M. Penafiel and R. Meister. 1991.
      Delineation of electric and magentic field effects of extremely low freuency electromagnetic
      radiation on transcription. Biochem. Biophys. Res. Comm., 174: 742-749.

g. Electromagnetic Field-Induced Effects on the Steady State Levels and Rates of Transcription of Particular mRNA Species.

SUMMARY

Exposure of HL-60 and Daudi cells to 60 Hz magnetic fields was done to investigate possible alterations in steady state levels and rates of transcription of specific RNAs. Steady state levels for the c-myc, and β-actin mRNAs and the 28 S rRNA were unchanged in exposed HL-60 cells. A slight, but highly variable enhancement for c-myc was found in field-exposed Daudi cells. A nuclease protection assay, to reveal field-induced changes in rate of transcription for specific RNAs showed no changes in HL-60 for either c-myc or β-actin mRNAs, but an approximately 40% enhancement in rate for the 45 S pre-rRNA.

INTRODUCTION:

In the previous section we demonstrated an enhancement in the rate of transcription of total cellular RNA produced when cells were exposed to 60 Hz electric fields. Work by others has focused on alterations in the expression of specific mRNAs by electromagnetic fields. Some of the results reported are remarkable in their rate and magnitude.

Goodman and associates (1) originally demonstrated dramatic changes in transcription of specific mRNAs by applying ELF fields to preparations of dipteran salivary glands. Induction of novel RNA puffing patterns on specific bands of the polytene chromosomes resulted. Subsequently, the same group has reported, in the human cell line HL-60, changes up to four-fold in the steady state levels of particular mRNAs within 20 m of the onset of ELF fields (2,3). Since their measurement is of the relative total amount of an mRNA, these data do not indicate whether increased transcription, decreased degradation, or both effects are responsible for the increase.

As dramatic as are the results reported by Goodman *et al.*, they have proven difficult to replicate. Our efforts to document field-induced changes in steady state levels of specific mRNAs are documented are herein described. We employed both Northern blotting techniques, to determine changes in the steady state levels of an mRNA, and a novel nuclease protection assay, to gauge relative rates of transcription for particular genes. Most of the work was done with the HL-60 line used by the Goodman group, but some work has also employed the Daudi human lymphoma cell line. Results from the HL-60 work have been submitted for publication.

METHODS:

Cell Cultures: HL-60 and Daudi cell lines were maintained as described in Methods of Section Ic.

Northern Blot Analysis: After exposure, total cellular RNA was isolated and an electrophoresed. After electrophoresis RNAs were transferred and fixed to nylon membranes. The membranes were prehybridized for 3 h at 44°, and then incubated for 18 h with ^{32}P-labeled cDNA probes for c-myc, β-actin, or 28 S ribosomal RNA. After hybridization the membranes were washed to remove any unbound probe, dried and autoradiographed.

Nuclease Protection Assay for Transcription Rate Analysis: Relative rates of gene-specific transcription were determined by quantification of the amount of pulse-labeled nuclear RNA protected from RNase degradation by a cold hybridization probe. Briefly, HL-60 cells were exposed to ELF fields for 90 m, and pulse-labeled for the final 15 m with ^{3}H-uridine at a concentration of 20 μCi/ml. Cells were harvested, lysed in 0.55 NP-40 lysis buffer, and nuclei were pelleted. Nuclear RNA was isolated from the nuclei by lysis in 0.5% SDS, 20 mM HEPES (pH 7.5), 5 mM $MgCl_2$, 1 mM $CaCl_2$. After partial alkaline hydrolysis, the labeled nuclear RNA was hybridized overnight with cold gene-specific probes to either c-myc, β-actin, or 45 S RNA. Following hybridization, the RNa was treated with S_1 nuclease, the protected RNA was precipitated with ethanol and collected on nitrocellulose filters. Filters were quantified by scintillation counting.

Electromagnetic Field Exposure: The solenoid exposure system described in Section Ig was employed for work with HL-60 cells. Ten Gauss, 60 Hz fields were applied for up to 90 m. Some exposures of both HL-60 and with the Daudi cell line were done with the Helmholtz system described in Section Ic; 60 Hz magnetic fields of 10 and 100 μT were used.

RESULTS AND DISCUSSION:

Effects of Electromagnetic Fields on Steady State Levels of Specific mRNAs: RNAs extracted from HL-60 cells exposed to the 10 Gauss field for 20, 60 and 90 m were examined for alterations in steady state levels of the c-myc and β-actin mRNAs, and for the 28 S rRNA. Densitometric analysis of autoradiographs prepared after Northern blotting techniques showed no significant, exposure-related change in the steady state levels of any of the three RNAs.

No change in the level of the 28 S rRNA was anticipated, since other experiments, reported in Section Ig, indicated that increased transcription was coupled with increased rates of degradation under these exposure conditions. Possible enhancements of the steady state levels of either the c-myc or β-actin mRNAs, however, were anticipated since both of these messages had been shown to be substantially enhanced by Goodman et al.. Since the 10 Gauss field used for our work was considerably higher than that employed by Goodman et al., additional exposures of the HL-60 cells were done with 10 and 100 μT, 60 Hz fields provided by Helmholtz coils. Under these conditions, designed to replicate their exposure parameters, results of Northern blot analysis still showed no significant differences in steady state RNA levels as the result of exposure.

An additional series of exposures, using the Helmholtz coil system and 100 μT, 60 Hz fields, was done using the Daudi human lymphoma cell line. Densitometric analysis performed for the c-myc mRNA only revealed a field-dependent enhancement of steady state mRNA levels. After 60 to 90 m of exposure, steady state levels of c-myc were 20-30% higher in exposed cells relative to matched controls. Unfortunately this enhancement was both small and quite variable, so that, statistically, it was with little significance.

We have, thus, been unable to replicate the rapid, dramatic increases in steady state levels of specific mRNAs reported by the Goodman group. To our knowledge there has been no replication of this work by any other research group. The only enhancement of steady state mRNA levels we have observed was in Daudi, not HL-60 cells, and this was minimal relative to the published data for HL-60. A curious aspect of the Goodman results is that not only readily alterable genes, such as c-myc are affected, but also "house-keeping" genes such as β-actin, and cell cycle-dependent genes, such as histone H2B, are equally affected. How a field would produce these uniform sorts of effects for genes that are very differently regulated is unclear.

Effects of Electromagnetic Fields on Transcription Rates of Specific mRNAs: Northern blot analysis provides indications of steady state levels of particular RNAs, but cannot distinguish whether any changes in such levels come from altered transcription, altered degradation, or both. The nuclease protection assay was used to examine HL-60 cells for field-induced changes in the rate of transcription of specific mRNAs. Results of this analysis are shown in Table 4.I. After a 90 m exposure to the 60 Hz, 10 Gauss field there were no detectable differences in the transcription rates for the c-myc or β-actin genes. An increase of approximately 42% was noted, however for the transcription of the 45 S pre-ribosomal RNA.

Relative Transcription Rates		
c-myc	β-actin	45 S pre-rRNA
1.00 ± 0.08	1.08 ± 0.10	1.42 ± 0.12

Increased transcription rate of the 45 S pre-ribosomal RNA was anticipated from the pulse label studies reported in Section Ig. The absence of enhancement in transcription rates for either c-myc or β-actin mRNAs in field-exposed HL-60 cells is reasonable in view of the results from Northern blot analysis for these mRNAs. The combination of results from the nuclease protection and the Northern blot assays suggest that: 1. Rates of rRNA transcription are sensitive to electromagnetic field exposure; 2. Rates of specific mRNAs may be little affected by field exposure, and; 3. Variations in field-induced effects on RNA patterns may occur among different cell lines. Clearly, additional data must be gathered, from other laboratories, before the issue of field-induced alterations in RNA transcription or steady state levels can be resolved.

REFERENCES Ig:

1.   R. Goodman, C. A. L. Bassett and A.S. Henderson. 1983. Pulsing Electromagnetic Fields Induce Cellular Transcription. Science 220: 1283-1285:

2.   Goodman, R., L. X. Wei, J. C. Xu, and A. Henderson. 1989. Exposure of Human Cells to Low-Frequency Electromagnetic Fields Results in Quantitative Changes in Transcripts. Biochimica Et Biophysica Acta 1009: 216-220.

3.   Wei, L. X., R. Goodman, and A. Henderson. 1990. Changes in levels of c-myc and histone H2B following exposure of cells to low-frequency sinusoidal electromagnetic fields: evidence for a window effect. Bioelectromagnetics 11: 269-272.

II. In Vivo Experiments: Effects in Developing Embryos of Applied Electromagnetic Fields

a. EMF-Induced Teratogenic Effects

The fear that low level, low frequency electromagnetic fields might adversely affect early embryonic development has led to a great deal of speculation about the safety of environmental exposure to such fields. Power lines, household appliances, and video display terminals are but a few examples of electromagnetic field sources that have become suspect as potential health hazards. Epidemiological studies of spontaneous abortion in humans exposed to these fields are extremely complicated to interpret because of control sample problems and a variety of confounding factors. Laboratory studies on mammals are relatively expensive, and, consequently, seldom are able to achieve statistically significant results because of limited sample size.

The idea that developing chick embryos in the early stages of incubation might serve as a model system for investigating the possibility that there is a danger associated with exposure to extremely low frequency (ELF) magnetic fields was examined first in the early 1980's (1) and has stimulated much research during the intervening years. A number of these subsequent chick embryo studies have corroborated the teratogenic effects of 1-μT to 10-μT magnetic fields; (1,2,3,4,5,6,7,8) however, have not been able to replicate this sensitivity of chick embryos to electromagnetic fields during their early development. (9,10,11,12) We are left with the perplexing question of why some laboratories obtain robust results demonstrating sensitivity of the developing embryo to electromagnetic fields, yet other labs demonstrate no effect at all. "Project Henhouse" (13) a six-laboratory replication effort, was established to decide the issue, but was inconclusive. Our own studies were aimed at attempting to resolve this issue and to probe the character of the interaction of electromagnetic fields with complex biological systems.

EXPERIMENTAL APPROACH

Developing white leghorn chick embryos (obtained from a commercial supplier, Truslow Farms in Chestertown, MD) were exposed to EM fields during the first 48 hours of incubation. The majority of the studies were carried out with three waveform types:

(1) 1-μT peak intensity pulses of 500-μs duration, 2 μs rise and fall times and a pulse repetition frequency of 100 Hz;

(2) 4-μT amplitude 60-Hz frequency sinusoidal waves; and

(3) 6.4-μT peak-to-peak 60-Hz sawtooth waves (rise time $\approx$ 16 ms, decay $\approx$ 1 ms).

The exposure systems duplicated those of the Henhouse experiment. In fact, two

of our exposure systems were actually used in one of the six Henhouse laboratories (EPA, Maryland site). Two additional exact replicas were built. As in the Henhouse experiment VWR Model-6000 water-jacketed incubators were used; however, to avoid any stray fields possibly arising from the coiled heater element located below the water jacket at the bottom of the incubator, the water was heated externally using RTE Model-110 FRC Bath/Circulators. The incubators were used interchangeably and randomly for "exposed" and "sham" configurations.

After the 48- hour incubation, the embryos are sacrificed and examined histologically for indications of abnormal development. The criteria used are those established for the Henhouse effort.(14) This evaluation was performed under blind conditions. Eggs were first examined for fertility and the embryos were determined to be live or dead. Live embryos were examined for abnormal morphologies. Embryos were considered to be abnormal if they differed markedly from the Hamburger and Hamilton (15) 48-hour developmental stages. Malformations were classified as cephalic nervous system, truncal neural tube, heart, blood vessels, and somites. Of these only the first two occurred with any significant frequency. Indeed over 90% of those embryos (both control and exposed samples) that were characterized as abnormal exhibited truncal neural tube malformations (as well as possibly other abnormalities).

OBJECTIVES

Three main objectives drove the specific experiments that were carried out in these studies. The first objective of the study was to try to provide a definitive answer to the question

Do low-level ELF electromagnetic fields (EMF) have any effect on the development of chick embryos? In particular, are there teratogenic effects?

The second aspect of the work attempted to address the question
Why has there been such variability in the observation of field-induced teratogenesis among the laboratories that have carried out similar studies?

The third goal was to attempt to discover some link between the observation of EMF-induced avian teratogenesis based on morphological abnormalities and to a biochemical effect at the cellular level. We shall treat these issues in order.

RESULTS

Based on exposure to pulse, sinusoidal-wave and sawtooth-wave EMFs, the answer to the first of the questions posed above is clearly affirmative. A two-year study of exposure to pulsed EMFs involving roughly 1600 eggs from 5 different flocks has shown nearly a doubling of the fraction of abnormally developed embryos when the incubating embryos are exposed to the EMF (Table 1.II below.). The statistical

significance of this result is characterized by a P-value less than 10^{-5}. Less extensive studies using sinusoidal and sawtooth EMFs led to the same conclusion with similarly persuasive statistical confidence levels--P $\approx 10^{-3}$ and P $\approx 10^{-6}$ respectively.

Table 1.II. Summary of the results for different waveforms.

EMF Waveform	Sham-exposed embryos			Field-exposed embryos		
	Live	Abnormal	Percent	Live	Abnormal	Percent
100-Hz 1-μT 100-μs Pulse	831	96	11.6	769	158	20.5
60-Hz 4-μT Sinusoid	222	19	8.6	163	22	13.5
60-Hz 6.4-μT Sawtooth	290	20	6.9	253	34	13.4

REPLICATION DIFFICULTIES

The answer to why there has been such variability in the observation of EMF-induced teratogenesis among different experimenters is complex; there is not a single dominant factor. Among the important elements are (a) a genetic predisposition to exogenous stress-induced abnormal development that is present in some fraction of the chicks produced by a given flock; (b) flock-to-flock variability; (c) seasonal variability; and (d) exposure to stresses other than EMFs, *e.g.*, heat, that lead to abnormalities in susceptible embryos. The situation is quite similar to that in which abnormal development is caused by the administration of chemical agents.

A scrutiny of our own results over the nearly two-year duration of the pulsed-EMF experiment provides significant insights. Our early experiments (February-April, 1990) indicated a robust teratogenic effect from exposure to pulsed electromagnetic fields (PEMFs); with the percentage of abnormal embryos in the control sample (206 live embryos) approximately equal to 11% of the live embryos, abnormal embryos in the exposed sample (204 live embryos) were approximately 26%. This agrees quite well with the two "Henhouse" laboratories that observed a significant increase in the rate of abnormal developments induced by electromagnetic field exposure. At this point it seemed that we would simply reinforce the conclusions of those groups without achieving any new insights. However, repetitions of these experiments during several distinct intervals over the following 20 months shows that the picture is more complicated.

One indication of complicating factors in these experiments appeared as a marked variation in the fraction of abnormal developments occurring in the control sample. Because of the small sample involved in a single run (usually either ten or twenty eggs), the number of abnormal embryos in the control group undergoes rather large fluctuations; indeed, with an average abnormality rate in the vicinity of 10%, a fluctuation of ± 1

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corresponds to a 50% or 100% variation. To smooth out the run-to-run variations, a moving average of order 15 was used. That is, for the a particular run, the nth, the pth order moving average of the control-sample abnormality rate, denoted by $<A_n^{(C)}>_p$, is obtained from the formula

$$<A_n^{(C)}>_p = \frac{\sum_{j=n-(p-1)/2}^{n+(p-1)/2} a_j^{(C)}}{\sum_{j=n-(p-1)/2}^{n+(p-1)/2} L_j} \, , \qquad (1)$$

where $a_j^{(C)}$ is the number of abnormal control-sample embryos out of a total of L_j live embryos in the jth run. In simple terms the average abnormality rate for the nth run was obtained by taking a weighted average of this run with the seven preceding and seven following runs.
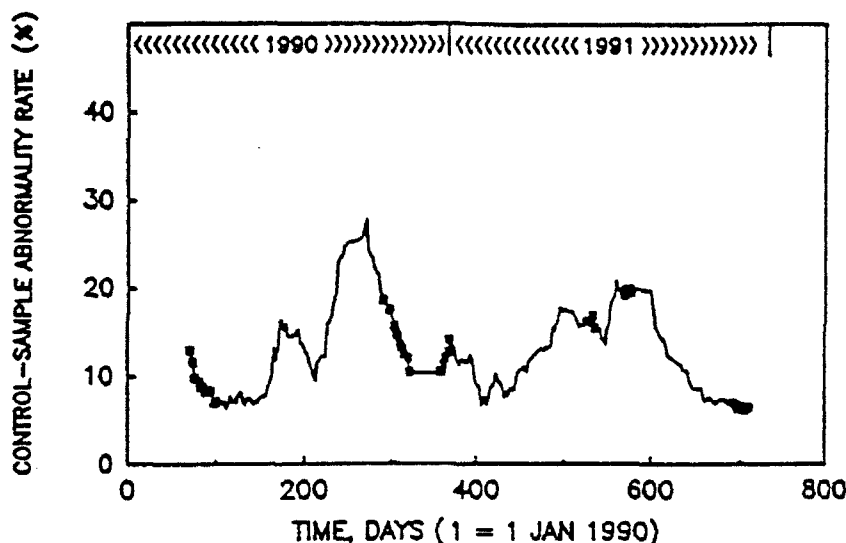
Figure 1.II The rate of abnormal development in untreated embryos measured from February, 1990 - January, 1992. PEMF exposure periods are shown by "■" superimposed on the curve.

This variation is shown in Figure 1.II. The data shown have been smoothed by a moving average of order 15. During the months May-October, 1990, it was observed that the control-group abnormality rate rose dramatically, achieving values (averaged over 15 runs) of 25 to 30%. The onset of the rise in the abnormality rate slightly preceded the supplier's putting a new flock of hens into service in mid-June. In mid-October, the control-group abnormality rate dropped, reaching again values in the neighborhood of 10% to 15%. Very shortly after this drop, a third flock was placed in

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service; this change produced no dramatic alteration of the control abnormality rate. A second campaign of exposing embryos to PEMFs was carried out during the period October 15, 1990 - January 5, 1991. This campaign involving 268 live embryos in the control group (124 from flock 2, 144 from flock 3) and 273 live embryos in the exposed group (125 from flock 2, 148 from flock 3) led to dramatically different results. The control group abnormality rates were essentially the same (about 12%) but the abnormality rate in the exposed sample had fallen to about 14%. There was no statistically meaningful effect. The results are given in Table 2.II.

A third campaign (June-July, 1991) using eggs from a fourth flock, resulted in yet a third type of result. The abnormality rate in the control sample jumped to about 25% (26/103) while that in the exposed group had returned to 27% (38/140). Again the small increase in the exposed group was statistically insignificant. When the untreated-embryo abnormality rate dropped in Fall, 1991 (to a value less than 10%), a fourth campaign was initiated. At this time the supplier put a fifth flock into service; this new flock exhibited untreated-embryo abnormality rates comparable to the reduced values in the earlier flock. This campaign (November-December, 1991) showed a substantial enhancement of the rate of abnormal embryo developments in the EMF-exposed sample (29/152, 19.1%) compared with the untreated sample (16/255, 6.3%).

This control-sample variability suggests a means for accounting for the results of the Henhouse experiment (laboratories B, C, D, I, K and M in Table 2.II), where only two of six laboratories found a robust positive effect. One explanation of this difficulty is statistical--in that experiment each laboratory reported the results of control and exposed samples of roughly 100 each. In principle, one should be able to combine the results from the six laboratories to obtain a single larger, and thus statistically more meaningful, experiment. The combined results indicate a field-induce increase in abnormality rates from roughly 15% (control) to 20% (exposed); the confidence level for these data is $P \approx 0.02$, consistent with our own results and supportive of the conclusion that electromagnetic fields do indeed have teratogenic consequences. Table 2.II presents a summary of the results from this work, from the participants in Henhouse, and from other experiments in which reasonable-size samples (on the order of or greater than about 100) were subjected to weak pulsed EMFs.

Table 2.II. Comparison of the results of various laboratories.

LAB CODE	Laboratory [Reference]	Percentage live embryos with abnormalities		
		Sham	Exposed	EMF-induced
A	CUA (This work), Campaign 4	6.3	19.1	12.8
B	Univ. Western Ontario [13]	6.4	20.6	14.2
C	Umeå, Sweden [13]	8.4	12.6	4.2
D	FDA, Rockville, MD [13]	9.7	22.2	12.5
E	CUA (This work), Campaign 1	10.7	26.0	15.3
F	CUA (This work), All runs	11.7	20.5	8.8
G	CUA (This work), Campaign 2	12.0	13.9	1.9
H	Univ. Kentucky/Mt. Sinai [11][a]	14.3	20.5	6.2
I	Madrid, Spain [13]	17.1	20.4	3.3
J	Univ. Rochester [10]	21.6	24.1	2.5
K	Univ. North Carolina, Chapel Hill, NC [13]	21.6	21.5	-0.1
L	CUA (This work), Campaign 3	25.2	27.1	1.9
M	EPA, Las Vegas, NV [13]	27.0	30.1	3.1

[a] Data are for 24-hour exposure. They are included since Martin [Ref. 7] has shown that there is no significant difference in the 24- and 48-hour-exposure results.

Of course, one should ask if combining the six data sets of the Henhouse experiment is a reasonable thing to do. If the project had been carried out as six identical replications of a specific experiment, then this pooling of the results would be a sensible procedure. However, for a variety of reasons, several of the participating laboratories used eggs that, in some way differed from the prescribed norm. In one case, locally supplied eggs from White Leghorn hens often exhibited an abnormality rate in excess of 50%, so eggs from a different strain of hens were used ("I" in Table 2.II). In a second case ("C" in Table 2.II), the eggs were obtained from a small flock especially set up for the study, creating the possibility that the normal genetic diversity that would be expected might be absent. In a third case ("K" in Table 2.II), eggs had to be flown in from an out-of-state supplier, increasing the possibility that they were subjected to unusual stresses, for example, heat. If the data from these three laboratories are removed from the tabulation, then the ratio of the abnormal live embryo developments to the total number of live embryos is $30/271 = 0.111$ in the control sample and $56/274$

= 0.204 in the exposed group, essentially identical to the values reported in this work. The important point here is that whether the "controversial" results are included or not does not alter the conclusion: *exposure to a weak electromagnetic field increases the rate of teratogenesis in developing embryos.*

In Table 2.II it is evident that the observed variability is quite a general phenomenon, not limited to our own experience or to the Henhouse project. It appears that flock-to-flock and seasonal variability is an important feature of the results. Again this can be confirmed by an elementary statistical analysis, although, the variability is evident from a glance at Figure 1.II. There is obviously a need to account for the wide variations in the percentage of EMF-induced abnormalities that is evident both in our data and in those of other workers as presented in Table 2.II. Except for the last two entries in the table (L and M) where we have tentatively attributed the unusually high abnormality rates in both the control and exposed samples to their being subjected to non-electromagnetic stresses (heat), the great majority (9 of 11) of the exposed-sample abnormality rates are in the vicinity of 20 to 25%. The differences in the EMF-induced abnormality rates among these thus arise primarily from the marked variations in the abnormal development rates in the sham-exposed samples.

HYPOTHESIS: GENETIC PREDISPOSITION

We therefore set out to examine the proposition that the fraction of abnormal embryos in the control group is anti-correlated with the fraction of field-induced abnormalities: the lower the rate of abnormal development in the control group, the higher is the field-induced abnormality rate. One possible explanation of this postulates that when a sample of developing embryos is exposed to an EMF, some fraction of the group will experience altered (abnormal) development as a result of that exposure. If the sample is characterized by a relatively high incidence of abnormal development in the absence of a field, then the *increase in the abnormality rate* due to exposure would be proportionately less. As a specific illustrative example, suppose that the effect of the field is to induce abnormality on 10% of the exposed embryos, and consider two samples of 100 eggs each, characterized by inherent abnormality rates of 10% and 20%. In each group, exposure to the field would affect 10 of the embryos. On the average in the first group, one of these embryos would already be destined to an abnormal development, while in the second group there would be two such embryos. The increase in the number of abnormal developments would thus be 9 in the first group (for an exposed-sample-abnormality-rate of 19%) and 8 in the second group (for an exposed-sample-abnormality-rate of 28%). This effect is much smaller than what is observed; the data seem to indicate a much stronger effect.

Our approach is to suggest that, within statistical variations, there is a relatively constant fraction of the embryo sample that is sensitive to abnormalities induced by external stresses such as heat or EM fields. Most of the abnormal embryo developments that appear in the control samples represent susceptible embryos that have been subjected

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to non-EMF stresses (e.g., heat). Those susceptible embryos that are not so affected prior to EMF-exposure will be rendered abnormal by the exposure. Thus the EMF-induced abnormality rate will be the difference between the fraction of stress-sensitive embryos and the control-sample abnormality rate. That is, the exposed-sample-abnormality-rate will be relatively constant from one experiment to another; fluctuations in the EMF-induced abnormalities are then attributed to variations in the abnormality rate (assumed to be non-EMF stress-induced) in the control-sample.

In testing this proposition, we have used several techniques for treating the data. The basic question is how to establish a "run control rate" (RCR) against which to compare a "run exposed rate" (RER). A run generally consists of simultaneously incubating approximately twenty to forty eggs, roughly half of which are exposed to the pulsed EMF, with the others constitute the sham-exposed or control sample.

Method 1: Direct Comparison

The RCR was taken simply as the abnormal development rate $A_n^{(C)} = a_n^{(C)}/L_n$. The abnormality rates for the exposed sample were then sorted into four groups based on these RCR values. The upper and lower limits of each of these bins were selected to make the number of exposed embryos in each bin as nearly the same as was possible. The results are shown in Figure 2.II. The rate of field-induced abnormalities is seen to decrease with increasing abnormality rates in the control sample. For the three lower control-sample abnormality rate groups (<5.5%, 5.5%-10.5% and 10.5%-18.5%) this decrease is accounted for almost entirely by the increase in the control-sample abnormality rate as the percentage of abnormal embryos in the exposed samples is essentially constant at about 18% or so. This constancy breaks down for the highest control-sample abnormality rate bin (>18.5%) where the exposed-sample abnormality rate rises to nearly 24%.
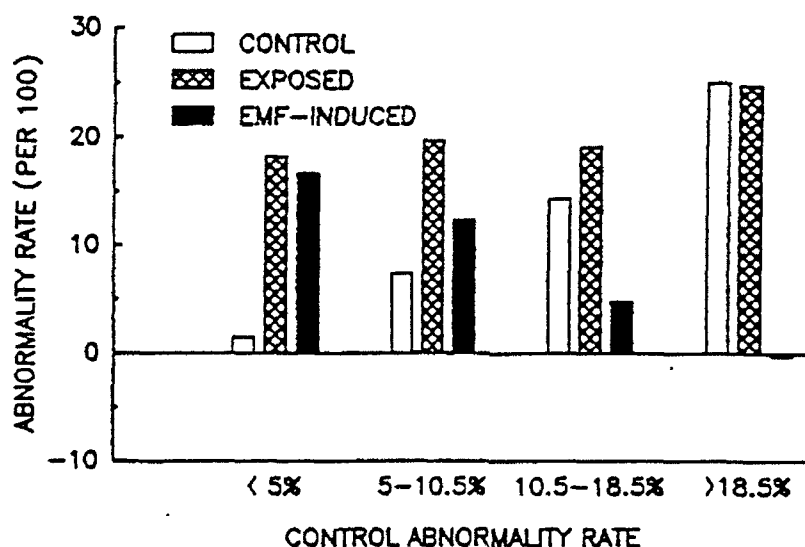

Figure 2.II. The rate of abnormal embryo development plotted versus the "run-control-rate."

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Method 2: Moving averages

Because of the small sample involved in a single run, the number of abnormal embryos in the control group undergoes rather large fluctuations; indeed, with an average abnormality rate in the vicinity of 1 in 10, a fluctuation of ± 1 corresponds to a 100% variation. To smooth out the run-to-run variations, a moving average of order 5 as defined in Eq. (1) was used, i.e., RCR = $<A_n^{(C)}>_5$. As above we sorted the data into four bins based on the RCR values, again choosing the boundaries of the bins to equalize the number of exposed embryos in each. The results are displayed in Figure 3.II. The overall trends are exactly those noted using the "direct comparison" method, although there are minor numerical differences in the specifics.
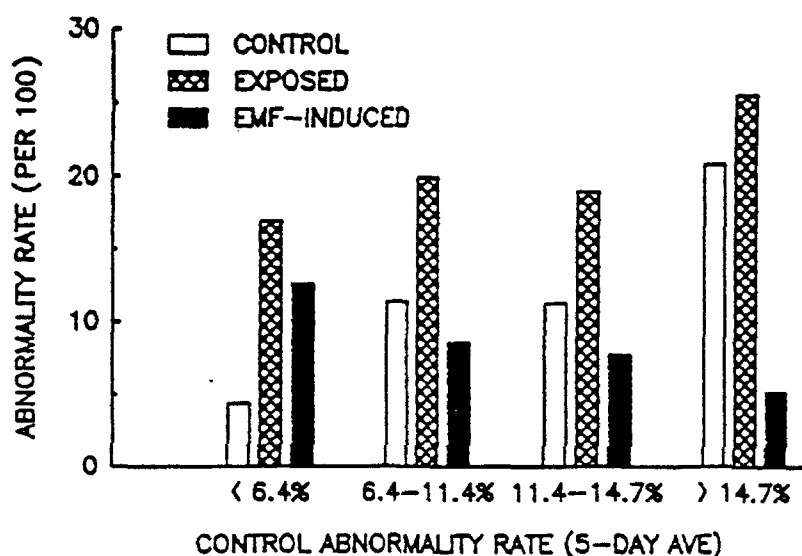
These results rather strongly support the hypothesis that motivated the approach in the first place. To test that the systematic variation obtained in methods 1 and 2 is not an artifact of the particular choices of bins, the analysis was repeated using (a) three bins chosen as above, (b) five bins chosen as above, and (c) four bins chosen arbitrarily as <10%, 10%-15%, 15%-20% and >20%. For these cases, the same systematic pattern is repeated.

Figure 3.II. The rate of abnormal embryo development plotted versus the run-control-rate smoothed by a moving average of order 5.

Method 3. Monte Carlo Assignments

Still a third approach was employed to verify the in a different way the observed variation. This approach also involved first sorting the individual runs into groups based on the RCR: Group 0, <5%; Group 1, 5-15%, Group 2, 15-25%, etc. These were then sorted into bins by the following procedure. For convenience we label the bins 01, 12, 23, etc. (the reason for this labeling will shortly become apparent). Each run in which the RCR fell in group 1 was placed in the 01 bin. For group 1 RCR values, a random number R in the interval 0 to 1 was selected and associated with that run. If R $\leq$ 0.5, the run was placed in bin 01; for R > 0.5, in bin 12. Similarly for group 2 RCR values, the same random number assignment procedure was carried out. Now for R $\leq$ 0.5, the run was placed in bin 12; for R > 0.5, in bin 23. The process is continued for

runs with RCR values in groups 3, 4 ... until all runs are placed in bins. This procedure was repeated five times and the results averaged to obtain rates of abnormal developments for the control and exposed groups for the different bins. These results are plotted in Figure 4.II. As was the case in the "direct comparison" and "moving average" methods, the pattern of a high rate of field-induced abnormalities when the control abnormality rate is low is seen.

DISCUSSION

To explore further the effect of the proportion of abnormal controls on the sensitivity of developing chick embryos to EM fields, we have in Figure 5.II plotted the data in the published literature along with our own results as a function of the observed rate of abnormal developments in untreated embryos. These data are listed in Table 2.II in order of increasing proportion of abnormal controls. The

Figure 4.II. The rate of abnormal embryo development using the "Monte Carlo" technique for sorting the results into bins describing the control-sample abnormality rate.

graph shows an obvious inverse correlation between the fractions of control and field-induced abnormally developed embryos. The laboratories reporting teratogenic effects had similarly low proportions of abnormal controls (below 15%), while the laboratories reporting no statistically meaningful effects from electromagnetic field exposure had high rates of abnormal embryos in the controls (above 20%).

Also interesting is the observation (still ignoring the Umeå and the CUA-2 data) that the total number of abnormals in the exposed group was relatively constant from one laboratory to another, ranging from about 20 to 25%. Exceptions to this are the results from our own campaign 3 and those from EPA-Las Vegas. In both of these cases suspected heat stresses have produced unusually high control sample abnormality rates (in excess of 25%).

The relative constancy of the total number of abnormals in the exposed group is intriguing and highly suggestive. It points to a genetic difference in chick embryos' susceptibility to electromagnetic fields; the idea is that only susceptible embryos will respond adversely to EMF exposure. This observation is consistent with standard notions in teratology and observed differences in human susceptibility to teratogens. Even embryonic exposure to thalidomide, one of the most potent human teratogens, during the
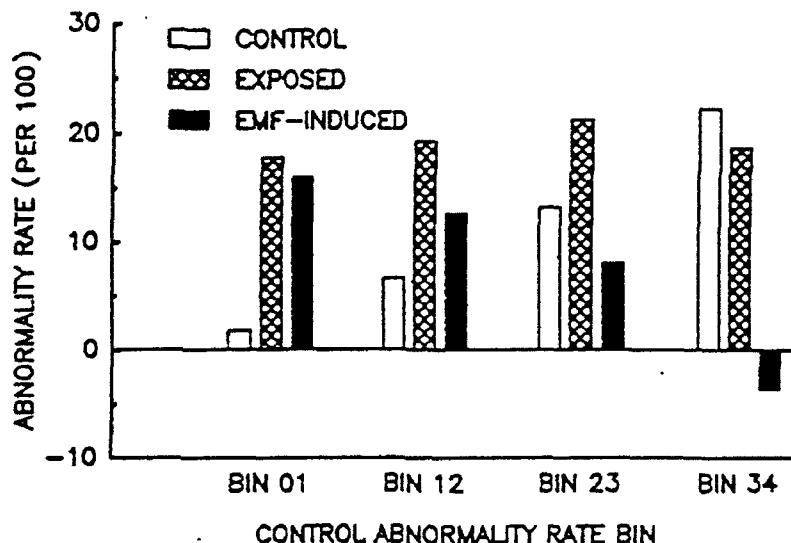
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critical developmental period results in a limb abnormalities in no more than 20% of the cases.(16,17) A review of chemical human teratogens demonstrates that abnormalities occur at rates less than (and usually substantially less than) 40%. Higher abnormality rates in the teratology literature occur only when the frequency of malformations is calculated from case reports. In these instances, the inherent bias of spontaneous reporting generally leads to overestimates as normal outcomes are less reliably reported.
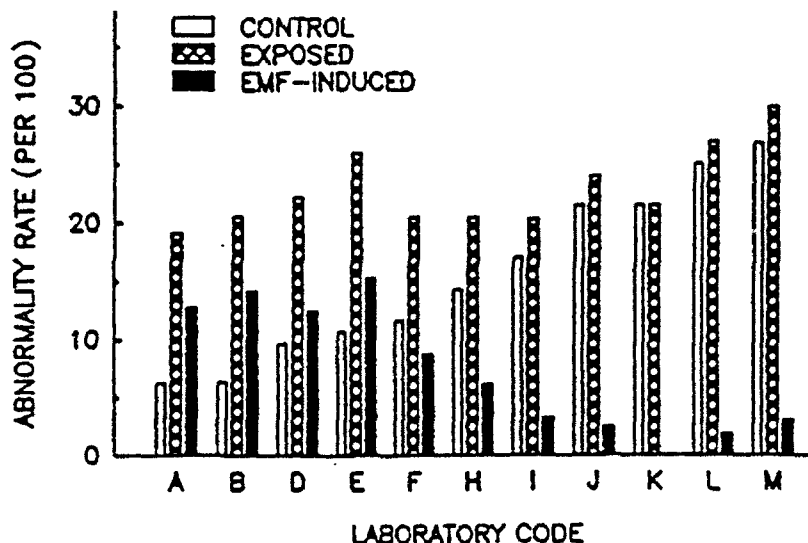
Figure 5.II. The rate of abnormal embryo development for eleven measurements at the various laboratories identified in Table 2.II.

The observation of a "ceiling" in the abnormal development rate suggests that the teratogen alone does not determine whether an embryo will be affected; an additional genetic susceptibility factor is indicated with malformations resulting from the interaction of the genotype and environmental factors. Thus differences in teratogenesis may be considered a genetic trait, with susceptibility or liability genes in either the maternal or fetal genotype.(18) Evidence supporting the role of genetic factors is provided by studies of the metabolism and teratogenicity of the anticonvulsant drug phenytoin. Phenytoin is metabolized to highly reactive arene oxide intermediates which are capable of covalent binding to embryonic or fetal macromolecules and nucleic acids, disrupting normal development. Individuals with low levels of epoxide hydrolase may accumulate toxic arene oxides, increasing their susceptibility to the toxic effects of epoxides. Measurement of fetal amniocyte epoxide hydrolase activity allows *prenatal* prediction of affected offspring, and provides confirmation that the teratogenicity of phenytoin is mediated, at least in part, by toxic intermediates produced during the biotransformation of phenytoin.(19,20)

A gene-teratogen interaction has also been identified with a non-chemical environmental stress. Inbred mice strains exhibit differing frequencies of heat-induced exencephaly, *indicating a genetic component of susceptibility.*[18] Such mice strains have also been used to demonstrate that the biological effect of an electromagnetic field can be genetically controlled--studies of the augmentation of receptor-bearing B lymphocytes

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by microwaves indicate that the susceptibility was controlled by a single dominant Mendelian gene.(21)

Adopting the hypothesis that EMF-induced teratogenesis requires genetic susceptibility on the part of the embryo offers the basis for ignoring the data obtained at Umeå, Sweden as part of the Project Henhouse effort. This is the only laboratory that did not use eggs obtained from a large commercial flock; we hypothesize that the low abnormality rates observed in this experiment reflects the fact that the small Swedish flock (25 hens compared with the order of 1000 in a commercial flock) was less genetically diverse than commercial flocks and, in this case, was characterized by a inherent susceptibility rate that is substantially lower than is obtained in a larger sample, more representative of the general population of this strain of hen.

b. EMF-Induced Biochemical Effects

We have also attempted to connect the observation of EMF-induced avian teratogenesis to a biochemical effect at the cellular level. It appears that a crucial component is the enhanced production of the inducible enzyme ornithine decarboxylase (ODC). ODC is a key enzyme in the biochemical pathways which are responsible for polyamine biosynthesis in mammalian cells. *In vitro* studies have been conducted on logarithmically-growing murine L929 fibroblasts which indicate an increased activity of ODC due to EMF exposure (see below in this report). In the chick embryo, ODC has been reported to peak twice within the first 24 hours of development,(22) after 15 hours of development (gastrulation) and after 23 hours (the onset of neurulation and early organogenesis).

We have compared the activity of ODC produced in embryos exposed to the 60-Hz 4-μT sinusoidal field described above with their sham-exposed counterparts after 15-hour and 23-hour incubation intervals. After incubation, for 15 or 23 hours, the embryos were excised by removing the vitelline membrane and blastoderm from the yolk. The vitelline membrane was then detached, thus isolating the blastoderm. The embryos were then observed under a dissecting microscope and separated according to the standard Hamburg and Hamilton(15) stages. In order to eliminate variability due to possible incubation before arrival at the lab, only embryos of the proper stage were kept for analysis. Thus, stage 3 embryos were kept for the 15-hour incubation time, and stage 5 embryos for the 23-hour incubation time.

A scalpel was then used to trim the area opaqua from the blastoderm so that only the area pellucida remained. This insured that only the embryo area proper was analyzed.(10) The embryos were then pooled according to incubator, with 4 to 6 embryos per tube. The tubes were centrifuged at room temperature frozen and then assayed. A total of approximately 280 embryos were used in the first study, and 160 in the second.

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The results indicate clearly that exposure to an EMF induces a bioeffect. For stage 3 embryos (15 hours incubation), ODC activity was significantly and consistently increased, with a ratio of activity in field-exposed embryos to that in the controls of 1.74 ± 0.36. For stage 5 embryos (23 hours incubation), the activity was significantly decreased, the exposed/control activity ratio being 0.65 ± 0.12. It is significant that the data show an increased activity for one time point and a decreased activity for the other. Preliminary work indicates that the time-dependent ODC activity curve is not simply increased or decreased by field presence, but rather that it is both increased and shifted in time.

The morphological and biochemical studies of developing chick embryos subjected to ELF electromagnetic fields, taken both individually and, especially, in combination, make it clear that the fields do indeed affect the early development of those embryos.

REFERENCES II:

1. J. M. R. Delgado, J. Leal, J. L. Monteagudo and M. G. Gracia, _Embryological changes induced by weak, extremely low frequency electromagnetic fields_, J. Anat. 134, 533-551 (1982).

2. J. Leal, A. Ubeda, M. A. Trillo, J. L. Monteagudo and J. M. R. Delgado. _Chick embryos orientation influenced by electromagnetic fields_, XXI General Assembly Annual Meeting, URSI, Florence, 1984.

3. J. Juutilainen, M. Harri, K. Saali and T. Lahtinen, _Effects of 100-Hz magnetic fields with various waveforms on the development of chick embryos_, Radiat. Environ. Biophys., 25, 65-74 (1986).

4. J. Juutilainen and K. Saali, _Development of chick embryos in 1 Hz to 1 kHz magnetic fields_, Radiat. Environ. Biophys., 25, 135-140 (1986).

5. J. Juutilainen, E. Läära and K. Saali, _Relationship between field strength and abnormal developments in chick embryos exposed to 50 Hz magnetic fields_, J. Radiat. Biol. 5., 787-793 (1987).

6. A. H. Martin, _Magnetic fields and time dependent effects on development_, Bioelectromagnetics 9, 393-396 (1988).

7. A. H. Martin, _ELF, PEMF's and development: why the controversy?_ Proceedings of the XXIII General Assembly of URSI, Prague, Czechoslovakia, August 28 - September 5, 1990.

8. A. H. Martin _Magnetic fields and time dependent effects on development_, Bioelectromagnetics 9, 393-396 (1988).

9. S. Maffeo, M. W. Miller and E. L. Carstensen, _Lack of effect of weak low frequency electromagnetic fields on chick embryogenesis_, J. Anat. 139, 613-618 (1984).

10. S. Maffeo, A. A. Brayman, M. W. Miller, E. L. Carstensen, V. Ciaravino and C. Cox, _Weak low frequency electromagnetic fields and chick embryogenesis: Failure to reproduce positive findings_, J. Anat. 157, 101-104 (1988).

11. B. F. Sisken, I. Fowler, C. Mayaud, J. P. Ryaby and A. A. Pilla, _Pulsed electromagnetic fields and normal chick development_, J. Bioelectricity 5, 25-34 (1986).

12. M. Sandstrom, K. H. Mild and S. Løvtrup, _Effects of weak pulsed magnetic fields on chick embryogenesis_, in "Work with Display Units", B. Knave and P-G. Wideback eds. (Elsevier Science Publishers, Amsterdam, 1986) pp. 135-140.

13. E. Berman, L. Chacon, D. House, B. A. Koch, W. E. Koch, J. Leal, S. Løvtrup, E. Mantiply, A. H. Martin, G. I. Martucci, K. H. Mild, J. C. Monahan, M. Sandström, K. Shamsaifar, R. Tell, M. A. Trillo, A. Ubeda and P. Wagner, _The effects of a pulsed magnetic field on chick embryos_, Bioelectromagnetics 11, 169-187 (1990).

14. E. Berman, Reproductive Toxicology 4, 45, (1990).

15. V. Hamburger and H. L. Hamilton, _A series of normal stages in the development of the chick embryo_, J. Morphol. 88, 49-92 (1951).

16. W. Lenz and K. Knapp, _Thalidomide embryopathy_, Arch. Environ. Health 5, 100-105 (1962).

17. K. Knapp, W. Lenz and E. Nowack, _Multiple congenital abnormalities_, Lancet 2, 725 (1962).

18. R. H. Finnell and G F. Charnoff, _Gene-teratogen interactions: An approach to understanding the metabolic basis of birth defects_ in "Pharmacokinetics and Teratogenesis. Volume II. Experimental Aspects _in Vivo_ and _in Vitro_", ed. H. Nau and W. J. Scott (CRC Pres, Boca Raton, 1987), Ch. 9.

19. B. A. Buehler, D. Delimont, M. van Waes and R. H. Finnell, _Prenatal prediction of risk of the fetal hydantoin syndrome_, New Engl. J. Med. 322, 1567-1572 (1990).

20. G. Koren, _Maternal-fetal Toxicology: A Clinician's Guide_ (Marcel Dekker, New York, 1990), Ch. 2.

21. C. J. Schlagel and A. Ahmed, _Evidence for genetic control of microwave-induced augmentation of complement receptor-bearing B lymphocytes_, J. Immunology 129, 1530-1533 (1982).

22. B. Löwkvist, O. Heby and H. Emanuelsson, _Essential role of the polyamines in early chick embryo development_, J. Embryol. Exp. Morph. 60, 83-92 (1980).

III. Dynamical Model of Dose Response Relationship

Data accumulated over the past decade or so imply that the dose-response features of biological systems exposed to ELF electromagnetic fields (EMFs) are unusual. Windows--ranges in which the system exhibits heightened sensitivity--have been reported both for field amplitude (or power) and the duration of periodic exposures. Examples of amplitude windows include electromagnetic field augmented transcription in HL60 cells exposed to 60-Hz fields,(1,2,3) field-induced abnormalities in developing chick embryos exposed to a 16.7-Hz sinusoidal electromagnetic field (4), and the field-stimulated enhancement in the growth of bone mass in turkey ulnae which display a maximum in bone growth versus average induced power for one-hour-per-day, five-day-per-week eight-week exposures to pulsed EMFs (5). We have developed a theoretical framework in which these amplitude windows are shown to arise as a natural consequence of the transient response of biological systems to externally applied electromagnetic fields.

TRANSIENT BIOCHEMICAL RESPONSE

Under certain exposure conditions the effect of extremely low-frequency (ELF) applied electromagnetic fields on certain measured properties of a biological system is transient (1,6,7). The system's response, determined by some well-defined endpoint, first rises following the switching on of the field, reaches some maximal value, and then decays, ultimately approaching some steady-state value (which may be the original equilibrium or basal value). We demonstrate below that this behavior naturally leads to the existence of maxima in the system's response (3,4,5,8) when regarded as a function of the exogenous field strength. These maxima arise in situations where the measurements involve observing the transient response at a fixed exposure time following the switching-on of the exogenous field.

As an example, suppose that we are monitoring the concentration of some particular species of messenger RNA as a function of time following the switching on of the field at $t = 0$. We suppose that, as a function of time, the mRNA concentration initially rises to some maximal value and then decays to a steady-state level (the exogenous field remaining on). Let us further suppose that the system's response depends on the strength or power density of the incident field such that increasing the strength both:

  (1)   increases the maximal value of the system's response (the peak is higher); and
  (2)   accelerates the response (the peak occurs sooner after switching on the field).

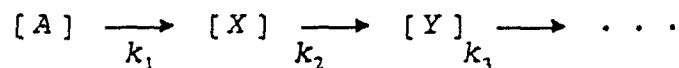Under such conditions the responses associated with three different field strengths will look something like the curves shown in Figure 1.III.

Next, consider that rather than being monitored as a function of time, the mRNA (or any other suitable endpoint) is measured only for some fixed exposure time T following the initial switching on of the field. Such an experiment might be designed to probe, for instance, the

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response of the system to the exposure for a time = T as a function of the field strength (irradiating power density). For the curves shown in Figure 1.III, the time = T exposure data would be represented by the bar graph in Figure 1.III. There is apparently a region of maximal sensitivity even though our assumptions above would seem to legislate explicitly against such behavior.

Observe that this conclusion rests on three, perhaps-not-too-unreasonable assumptions--(1) there is a transient response to an impressed field; (2) a maximal response that increases with increasing strength of the irradiating field; and (3) a peak location that shifts to earlier time as the field strength is increased. We have developed a simple physical model that predicts this kind of behavior, and although it is surely much too crude to portray accurately a process as complex as, for instance, RNA or protein synthesis, it can nevertheless prove useful as a guide for designing and as a vehicle for interpreting electromagnetic field exposure experiments.

Figure 1.III. (top) Typical transient response to EMF switched on at $t=0$; (bottom) apparent power window resulting from observations at $t=T$.

We have shown that certain window effects can be accounted for by recognizing the transient character of the response of the biological system. We have introduced a simple multi-step chemical reaction model that mathematically reproduces the major response features. We model the biochemical pathway as a series of reaction events, which we assume to be strongly biased in the forward direction. Schematically, this is represented as

$$[A] \xrightarrow{k_1} [X] \xrightarrow{k_2} [Y] \xrightarrow{k_3} \cdots$$

where [A] describes the reactant pool, [X], [Y] ... represent a set of reaction products that are synthesized sequentially and the k's are rate constants that describe the speeds at which the reactions progress.

The crucial assumptions of the model are that the immediate effect of initiating exposure to EMFs is a sudden increase in one or more of the rate constants and that this (these) grows (grow) monotonically with the exogenous field strength. The consequences of this are a transient rise in the response (the concentration of one of the reaction products, say [Y]) following the switching on of the field at time $t = 0$ followed by a subsequent decay. Of
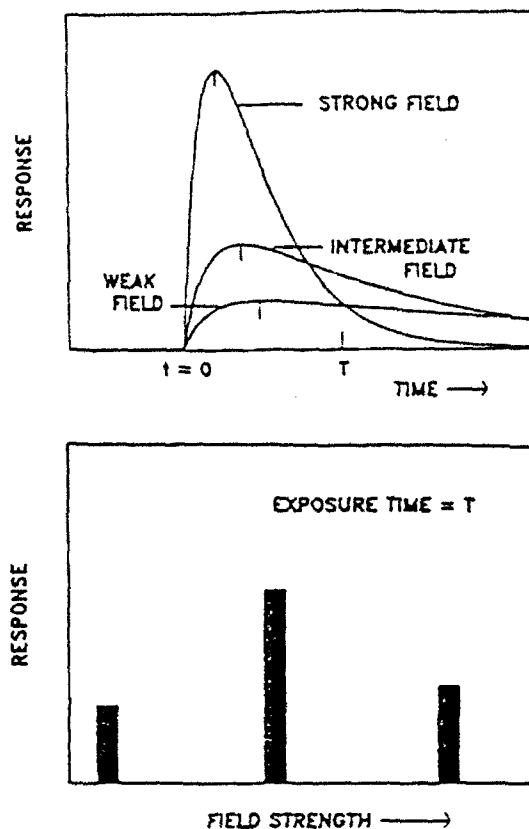
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course, we recognize that what we have described as a *single* reaction step undoubtedly consists of a number of complex individual processes. Our assumption is that among these processes there is one that is "rate-determining" so that the simplification shown above is meaningful. No qualitative changes in the model's predictions would result if additional steps were added to the sequence, although the equations would become more complicated and the exact shapes of the response curves would be altered.

MATHEMATICAL FORMULATION

We assume that the reactant pool is rapidly replenished, that back-reactions can be neglected, and that they are described by a set of linear first order differential equations

$$dx/dt = + k_1a - k_2x; \tag{1a}$$

$$dy/dt = + k_2x - k_3y. \tag{1b}$$

The equilibrium values (in the absence of any external electromagnetic fields) are easy to determine from $dx/dt = dy/dt = 0$:

$$x_{eq} \equiv x_0 = k_1a/k_2; \tag{2a}$$

$$y_{eq} \equiv y_0 = k_2x_0/k_2 = k_1a/k_3. \tag{2b}$$

We now hypothesize that switching on the electromagnetic field at $t = 0$ produces a "sudden" increase in k_2, that is

$$k_2 \rightarrow k_2^*$$

with this change occurring on a time scale that is short compared with the inverse reaction rates. Using this, it is now a straightforward matter to solve the differential equations to determine the concentrations as functions of time:

$$x(t) = \frac{k_1a}{k_2^*} [1 + \frac{\Delta k}{k_2} e^{-k_2^*t}]; \tag{3}$$

and

$$y(t) = \frac{k_1 a}{k_3} + \frac{k_1 a}{k_3 - k_2^{\bullet}} \frac{\Delta k}{k_2} [e^{-k_2^{\bullet} t} - e^{-k_3 t}] \qquad (4)$$

where

$$\Delta k = k_2^{\bullet} - k_2. \qquad (5)$$

The quantities $x(t)$ and $y(t)$ are plotted in Figure 2.III for three values of the altered rate constant k_2^{*}.

The parameters for the plots are given in a set of reduced units. From the differential equations (1), it is clear that the rate constants have units of $(time)^{-1}$. It is therefore sensible to choose one of them as defining the basic time unit: we do this by setting $k_1 \equiv 1$. In a similar fashion, we take a as the basic concentration unit, i.e., $a \equiv 1$. Equivalently, we may regard all rate constants and concentrations as being given by ratios with k_1 and a respectively:

$$k_j \longrightarrow k_j/k_1, \quad x \longrightarrow x/a$$
$$\text{and } y \longrightarrow y/a$$

Similarly times can be regarded as products with k_1:

$$t \longrightarrow k_1 t$$

For the plots in Figure 2.III we have taken $k_2 = 1$, $k_3 = 10$, and $k_2^{*} = 3$, 10 and 30.

Figure 2.III. The evolution of $x(t)$ and $y(t)$ after switching on an EMF at $t=0$ from Eqs. (3) and (4) for three values of k_2^{*}.

Observe from Figure 2.III that, independent of the value of k_2^{*}, the long-time steady-state field-on concentration is the same as the zero-field concentration--the basal level, i.e., $y(\infty) = y(0)$. The transient character of the response in this model is relatively easy to understand. The initial increase in $y(t)$ arises from the enhanced rate k_2^{*}; the drop off at long times (back to the equilibrium value) comes from the depletion of the precursors x. Note in particular that
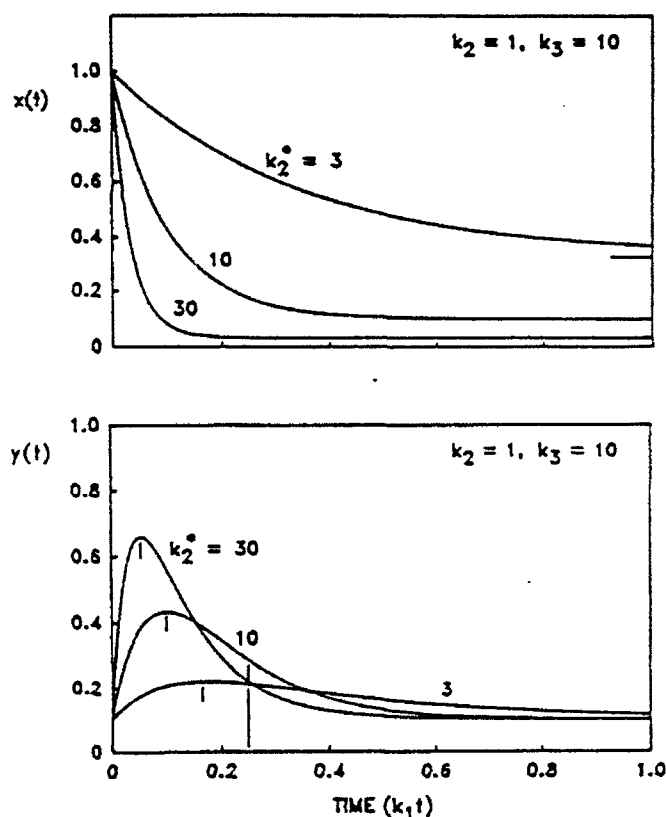
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(1) the position of the maximum of y(t) marked by " | " on the plots moves toward $t = 0$ with increasing k_2^*, and

(2) the peak height increases with increasing k_2^*.

APPARENT POWER WINDOWS

If we were now to assume that Δk increases with irradiating field strength (or power density) that is, the change in the rate constant governing the [X] → [Y] reaction increases with the electromagnetic intensity, *then all features of the transient responses would be qualitatively reproduced by this simple model.*

Note that for the parameters chosen for this sample calculation, an exposure time $t_x = 0.25$ (vertical line on Figure 2.III) leads to a maximum in the response considered as a function of k_2^*, field strength or power density, i.e., a so-called power window. This is illustrated on the bar graph in Figure 3.III. For shorter exposure times (Figure 3.III illustrates the case of an exposure time $t_x = 0.12$) the maximum shifts to higher power densities. Within the context of the linear reaction model, fixed observation times will *always* lead to a maximum in the response regarded as a function of the field strength, with the location of the maximum a function of the exposure time.

The location in time of the peak that is observed in the response of the system can be determined by differentiating Equation (4). The result is

$$t_{max} = \frac{1}{k_3 - k_2^*} \ln (k_3 / k_2^*) \tag{6}$$

and the magnitude of the maximum response is given by substituting this back in Equation (4). Defining $y_{max} \equiv y(t_{max})$ leads to

$$y_{max} = \frac{k_1 a}{k_3} + \frac{k_1 a}{k_3 - k_2^*} \frac{\Delta k}{k_2^*} \left[(k_2^* / k_3)^{k_2^* / (k_3 - k_2^*)} - (k_2^* / k_3)^{k_3 / (k_3 - k_2^*)} \right]. \tag{7}$$

Both t_{max} and y_{max} are plotted as functions of the changed rate constant k_2^* in Figure 4.III. Observe that y_{max} saturates as k_2^* is increased so that the peak height cannot be used as a measure of the change in k_2. That is, for large values of k_2^* the peak height does not change although the location of the peak does shift to shorter times. This saturation occurs when k_2^* has become so large that it no longer limits the production of [Y], which is controlled exclusively by the availability of the precursor [X].

The change in k_2 produced by switching on the field can be found from the initial rate of change of the response. Expanding Equation (4) about $t = 0$ shows that y increases linearly for short times:

$$y(t) - y_0 \equiv \delta y(t) \approx \frac{k_3}{k_2} \Delta k \, t$$

$$(t << t_{max}).$$

It is clea. that the initial slope of the response is proportional to the magnitude of the change in k_2, that is to Δk.
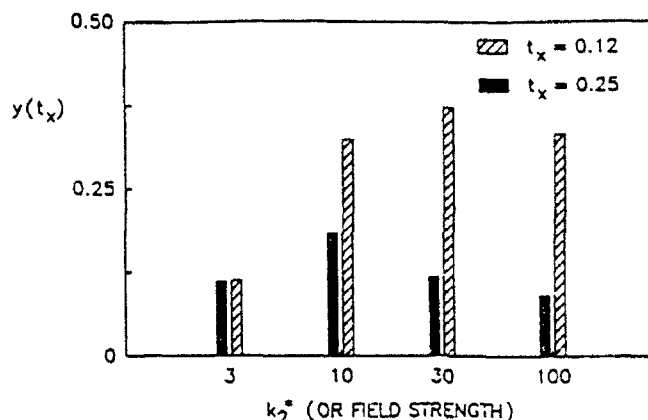
Figure 3.III. Amplitude windows resulting from observing the responses shown in Figure 2.III at $t_x =$ 0.12 and 025.
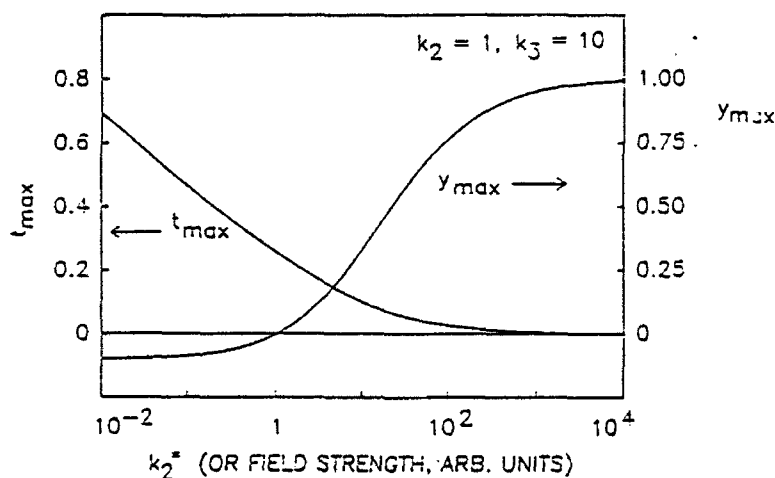
Figure 4.III. The dependence on field strength of the peaking time t_{max} and the maximum response y_{max}.

Note also the behavior of the response at long times. With the field remaining on, the system marker, y(t), returns to its equilibrium value at a rate determined by the *smaller* of the reaction rates k_2^* and k_3. Specifically, if one of the rates is much larger than the other, we have

$$\left[\frac{d}{dt} \ln \delta y \right]_{t \to \infty} \approx -k_< \qquad (k_< << k_>), \tag{8}$$

where $k_<$ $(k_>)$ is the smaller (larger) of k_2^* and k_3.

In the reaction model just described, the transient increase and subsequent fall of the measured marker was produced by assuming that the effect of the impressed field is to increase the production rate k_2. It is reasonable to inquire if a similar result can be derived if we assume that the effect of the field is to stabilize the product [Y], that is, to decrease its rate of degradation, k_3. The answer is that within the context of this elementary linear picture, it is not. If we had assumed, for example, that switching on the field at $t = 0$ causes k_3 to be suddenly

changed to some new value k_3^* ($< k_3$), then we would obtain for the response

$$x = \frac{k_1 a}{k_2} = \text{a constant, } i.e., \text{ no change,} \qquad (9)$$

and

$$y(t) = \frac{k_1 a}{k_3^*} [1 - (\frac{k_3 - k_3^*}{k_3}) e^{-k_3^* t}]. \qquad (10)$$

The concentration $y(t)$ simply rises monotonically from its initial level $k_1 a / k_3$ to an increased level $k_1 a / k_3^*$; there is no later return to the pre-exposure level.

THE REBOUND EFFECT

Lastly we note the effect of switching *off* the impressed field. Assuming that the effect of the electromagnetic field on the rate constant is reversible (which seems reasonable since we are considering weak fields), then the task is to solve again the differential equations for $t \geq t_0$ (the time at which the field is switched off) using k_2 rather than k_2^*. We consider first the effect of turning off the field *after the concentration y(t) has returned to its pre-irradiation equilibrium value*. This is shown for $k_2^* = 30$ in Figure 5.III; *y(t)* drops below the steady-state value immediately following the cessation of the irradiation. The concentration reaches a minimum, and then slowly return to its equilibrium value. The "rebound" is easily understood as resulting from the suddenly decreased rate of [Y] production ($k_2 < k_2^*$) coupled with the depleted population of the species [X].

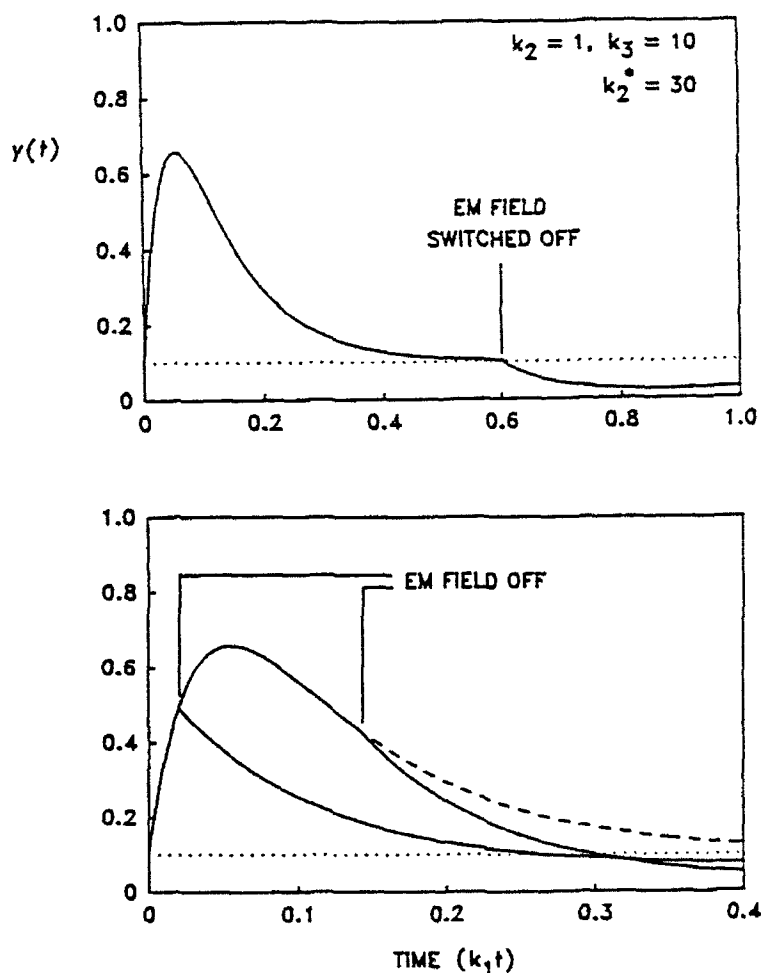Figure 5.III. *y(t)* versus *t* for an EMF switched on at *t=0* and off at *t=.6* (top) and at 0.03 and 0.14 (bottom). The broken curve gives the fields-on behavior.

If the exogenous field is switched off at a time prior to the system's response returning to its pre-exposure level, then the effect is a sudden decrease in the system's response $y(t)$ leading to an overshoot of the basal value, and, finally, to a slow return to this equilibrium value. This behavior is illustrated for two different "switch-off" times in Figure 5.III.

MODEL IMPLICATIONS

If the results of this theoretical analysis are generally applicable to experimental data, then several generalizations can be made. Studies to determine the effect of long term exposure to electromagnetic fields should examine the effects of relatively short, repetitively applied exposures in addition to the more usual duration-of-the-experiment exposures. Epidemiological data may well not correlate with the average field measured in the home or work place, because, as we have seen, the time duration of each separate environmental exposure and the time variations of the field strengths must be taken into account. If there are adverse health effects of EMFs, then frequency with which one enters and the duration of each stay in a region of electromagnetic exposure becomes important.

GENERALIZATION OF THE MODEL

The multi-step model has successfully described (at least qualitatively) the transient augmentation of transcripts observed when cell cultures are subjected to ELF fields. It can also "explain" the window effects observed in chick embryo experiments where delaying the imposition of the field until roughly 12 hours into the incubation process increases its teratogenic effect. However it is not sufficient to account for the amplitude window in the field-stimulated enhancement in the growth of bone mass in turkey ulnae reported by Rubin, McLeod and Lanyon.(5) Their results presented in Figure 6.III display a maximum in bone growth versus average

Figure 6.III. Percent change in cross-sectioned bone area for exposure to EMFs of different strengths.

induced power for one-hour-per day, five-day-per-week eight-week exposures to pulsed electromagnetic fields.

Motivated by that inadequacy, we have modified the multi-step model in a way that preserves the important conceptual features of the original model, but also offers an explanation for the data of Figure 6.III. The problem in applying the result as was done in those cases is that the measurements do not reflect the concentration of some species *measured at some specific instant of time*, but rather describe an accumulated effect--an increase in bone mass. Instead of a transient phenomenon that initially rises and then returns to its steady-state level, the change in bone mass might be expected to be proportional to the integral of the direct transient response:

$$\Delta m \propto u(t) \equiv \int_0^t dt' \, \Delta y(t') \qquad (11)$$

where Δm is the increase in bone mass and $\Delta y(t)$ is the transient bio-response (excess over control) that triggers the bone growth. Moreover the situation is such that although the measurements are made after a time t, the field is switched off at some time $t^* < t$. Assuming that the rate constant k_2 returns to its original value when the field is switched off, i.e., $k_2^* \to k_2$, the multi-step model along with equation (11) predicts no maximum as a function of field strength; for a given experimental time t, $u(t)$ increases monotonically with Δk_2. The simple multi-step chemical model must be modified if it is to be retained as a framework for explaining single amplitude windows in which an integrated effect is observed. Fortunately, a relatively simple modification to the model both preserves the qualitative character of the original model and accounts for the Rubin, McLeod and Lanyon result.

The multi-step model described above has as its basic assumption that only one of the rate constants (e.g. the transcription step) is affected by the electromagnetic field. We shall assume that switching on the field increases both the synthesis and degradation rates,

$$k_2 \longrightarrow k_2^* \quad \text{and} \quad k_3 \longrightarrow k_3^*,$$

and that k_2^* and k_3^* rapidly return to their original values when the field is switched off. It is a straightforward matter to solve the linear differential equations describing the sequential reaction scheme. For a field switched on at time $t = 0$ and switched off at time $= t^*$, the solutions for the concentration of [Y], $y(t \leq t^*)$ and $y(t \geq t^*)$, are then

$$y(t \leq t^*) = \frac{k_1 a}{k_3^*} [1 + \frac{\Delta k_3}{k_3} e^{-k_3^* t}] + \frac{k_1 a}{k_3^* - k_2^*} \frac{\Delta k_2}{k_2} [e^{-k_2^* t} - e^{-k_3^* t}] \qquad (13)$$

and

$$y(t \geq t^*) = \frac{k_1 a}{k_3} + B e^{-k_3(t - t^*)} + C e^{-k_2(t - t^*)}, \qquad (14)$$

where $\Delta k_3 = k_3^* - k_3$,

$$B = -\frac{k_1 a}{k_3 - k_2} \frac{\Delta k_2}{k_2^*} [1 - e^{-k_2^* t^*}],$$

and

$$C = -B - \frac{k_1 a}{k_3} \frac{\Delta k_3}{k_3^*} [1 - e^{-k_3^* t^*}] + \frac{k_1 a}{k_3^* - k_2^*} \frac{\Delta k_2}{k_2} [e^{-k_2^* t^*} - e^{-k_3^* t^*}].$$

These equations derived for the modified multi-step chemical model lead to interesting predictions. Figure 7(a).III shows the transient bioresponse for several exposure times (t^*). The reaction rate constants are chosen as: $k_1 = 1/60$, $k_2 = 1/15$, $k_3 = 1/6$, $k_2^* = 1.3$, and $k_3^* = 0.5$. Observe that the transient peak-and-decay feature of the original model is preserved, and that the concentration $y(t)$ decays more slowly if the field is turned off after the peak is reached.

Figure 7(b).III shows the situation for various field strengths and a fixed exposure duration t^*. Linear relationships between Δk_2^* and Δk_3^* and E were assumed to obtain these curves; specifically

$$\Delta k_2^* = 1.23 \, E \quad \text{and} \quad \Delta k_3^* = 0.333 \, E.$$

The actual values of the parameters used $(E; k_2^*, k_3^*)$ are $(0.2; 0.31, 0.23)$, $(1; 1.3, 0.5)$ and $(5; 3.75, 1.16)$; the other rate constants $(k_1, k_2,$ and $k_3)$ are the same as for Figure 7(a).III. Recall that the excess protein and thus, as discussed in connection with the data of Figure 1(c).III, the increase in bone mass produced by exposure to electromagnetic fields is approximately proportional to $u(t)$, the integral of $\Delta y(t')$ as given in equation (11). We have calculated this, i.e., $u(t)$, for the exposures shown in Figure 7(b).III. The integrals over t' are carried out for a total measurement time of 8 hours. The results are shown in Figure 8(a).III. In contrast to the situation in which only k_2 changes under the application of an external field, we note that here a maximum is indeed observed as a function of field strength. This is illustrated in Figure 8(b),III. The power window seen by Rubin, McLeod and Lanyon (1989) shown in Figure 6.III is consistent with the hypothesis that the exogenous field alters both k_2 and k_3. In Figure 9.III we show a fit obtained to these results.

This modified model also predicts a surprising result, if, for a given field strength, the increase in bone mass, i.e., $u(t)$, is examined for a variety of exposure intervals t^*. From Figure 7(a).III, it can be seen that a maximum in $u(t)$ (and thus implicitly a peak in the amount of
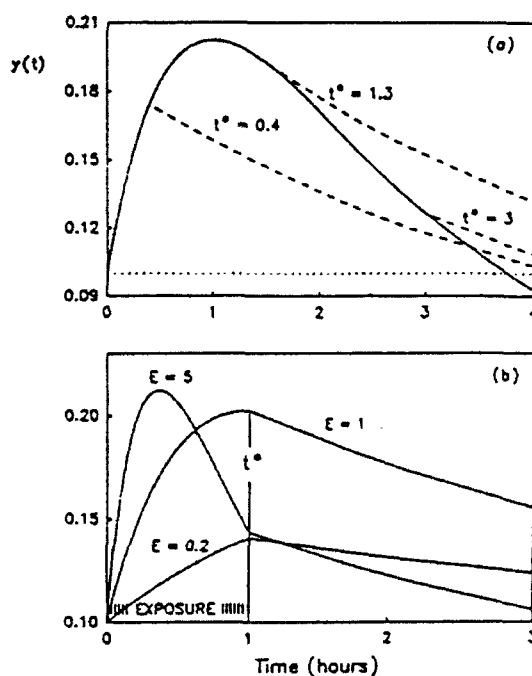

Figure 7.III. (a) The transient response to an EMF switched on at $t = 0$ and off at times t^*; (b) the response for $t^* = 1$ and three field strengths.

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protein produced by the translation process) as a function of t^* will be found. This means that there exists a certain optimum time duration of exposure that causes significantly larger bio-effects than are observed for either longer or shorter exposure times. For the constants used in the calculations above, it turns out that a one-hour exposure is most effective for achieving some bio-effect (either harmful or beneficial).

This is just the type of result that has recently been found by Ciombor, Aaron and Polk[1] who have observed that optimum cartilage growth is achieved by exposing animal subjects to electromagnetic fields for specific daily intervals of approximately two hours. Doses either shorter than or much longer than this interval produce less pronounced effects. Assuming the correctness of the modified chemical model it is easy to understand how this comes about. For a given field strength, the maximum area under the concentration versus time curve occurs when the field is switched off when the response $y(t)$ is at its peak. This causes the rate constants k_2^* and k_3^* to return to their original *smaller values*, thereby effecting a slower return to basal levels than would be the case were the field allowed to remain on.

DISCUSSION

To account for the range of amplitude-window data extant in the literature, we have had to conclude that the electromagnetic field affects both the rate of synthesis and the rate of degradation of metabolic products. The most important (as well as most surprising) implication of this result is the prediction that maximum bio-effect occurs at some intermediate (relatively short) time duration of the exposure (at any given field strength). This has implications in the design of both *in vivo* and *in vitro* experiments as well as in the analysis of epidemiological data.

If the results of this theoretical analysis are generally applicable to experimental data, then several generalizations can be made. Studies to determine the effect of long term exposure to electromagnetic fields should examine the effects of relatively short, repetitively applied exposures in addition to the more usual duration-of-the-experiment exposures.

Figure 8.III. The integral of the response for $t = 8$ h as a function of (a) t^*, and (b) field strength E.

Epidemiological data may well not correlate with the average field measured in the home or work place, because, as we have seen, the time duration of each separate environmental exposure and the time variations of the field strengths must be taken into account. If there really are any adverse health effects of electromagnetic fields, then the number of times a day that one enters and the duration of each stay in a region
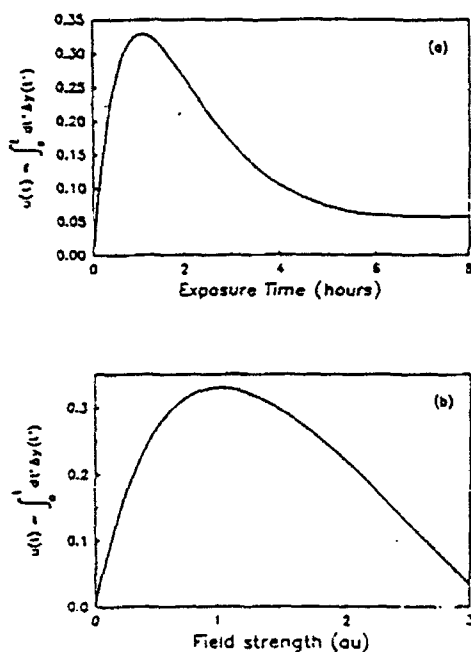
of electromagnetic exposure becomes important. A favorable situation occurs when one enters the field and stays there for a long time.
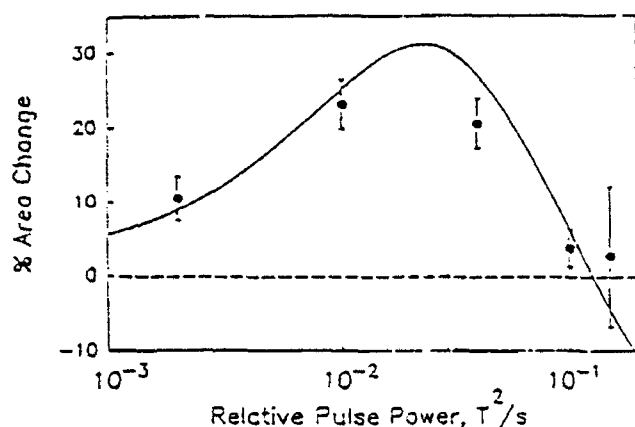

Figure 9.III. A fit of the data in Fig. 6 to the function $u(t)$ for $t = 8$ h and Δk_i^* and Δk_j^* linear in the relative power.

Both the original and the modified multi-step chemical reaction models represent tremendously simplified hypotheses describing the interaction of electromagnetic fields with biological systems. From a computational standpoint the assumption of linearity offers great simplification at the price of some generality. While this is certainly a limitation of the numerical predictions derived from the models, the spirit of the model is more aimed at developing a conceptual method for organizing thinking and designing experiments. Moreover, we assume that linear response provides a reasonable first-approximation in the early stages of exposure before adaptive bioregulation processes are activated. A second limitation is that the models offer no mechanistic basis for understanding the alteration of biological functioning. The basic hypothesis is that in some fashion the imposition of an electromagnetic field alters one or more reaction rates in the biochemical pathway. No detail is given of the enzymatic basis for this or of the nature of the field-system interaction that initiates the change.

However, models of this sort nevertheless are useful because they provide a framework for correlating a variety of experimental results. They imply a connection between features of experimental data (such as transient response and single amplitude windows), and consequently are amenable to experimental testing. This testing can be expected to lead to improvements in the understanding, describing and modeling of the bio-response.

REFERENCES

1.   R. Goodman and A. S. Henderson, _Sine waves enhance cellular transcription_, Bioelectromagnetics 7, 23-29 (1986).

2.   R. Goodman, L-X. Wei, J-C. Xu and A. S. Henderson, _Exposure of human cells to low-frequency electromagnetic fields results in quantitative changes in transcripts_, Biochem. Biophys. Acta 1009, 216-220 (1989).

3.   L-X. Wei, R. Goodman and A. S. Henderson, _Changes in levels of c-myc and histone 2B following exposure of cells to low frequency sinusoidal electromagnetic fields: evidence for a window-effect_, Bioelectromagnetics 11, 269-272 (1990).

4.   J. Juutilainen and K. Saali, *Development of chick embryos in 1 Hz to 1 kHz magnetic fields*, Radiat. Environ. Biophys. 25, 135-140 (1986).

5.   C. T. Rubin, K. J. McLeod and L. E. Lanyon, *Prevention of osteoporosis by pulsed electromagnetic fields*, J. Bone and Joint Surg. 71A, 411-417 (1989).

6.   R. Goodman, C. A. L. Bassett and A. S. Henderson, *Pulsing electromagnetic fields induce cellular transcription*, Science 22, 1283-1285 (1983).

7.   R. Goodman, L-X. Wei, J-C. Xu and A. S. Henderson, *Relationship of Transcript Quantity to Signal Amplitudes*, presented at the Eleventh Annual Meeting of the Bioelectromagnetics Society, Tucson, AZ, June 18-22, 1989.

8.   C. F. Blackman, L. S. Kinney, D. E. House and W. T. Joines, *Multiple power-density windows and their possible origins*, Bioelectromagnetics 10, 115-128 (1989).

9.   D. McK. Ciombor, R. Aaron and R. Polk R, *Sine wave interaction with in vivo model of bone development*, Proc. Bio. Repair and Growth Soc. 11, 11 (1991).

IV. The role of spatial and temporal coherence in the response of biological systems to electromagnetic fields

SUMMARY

Living cells exist in an electrically noisy environment. We propose that living cells are affected only by electromagnetic fields that are spatially coherent over their surface. The bc ʻ· idea is that a significant number of sensors at the cell membrane must be simultaneously and coherently activated (coincidence detection) to produce an effect on the biochemical functioning of the cell. This provides a solution to the so-called signal-to-noise dilemma whereby cells respond to extremely-low-frequency exogenous electromagnetic fields that are several orders of magnitude weaker than local endogenous fields associated with thermal fluctuations. However, like all physical detection systems, cells are subject to the laws of conventional physics and can be confused by noise. This suggests that if a spatially coherent but temporally random noise field were superimposed on the coherent signal, then at some value of the signal/noise amplitude ratio, the discrimination mechanism would be defeated, and the observed field-induced bioeffect would be suppressed. Experimental evidence on morphological abnormalities and ODC activity in developing chick embryos is consistent with this idea.

The association of biological effects at the cellular level with exposure to weak electromagnetic fields (EMFs) has remained a controversial subject despite nearly a decade of such reports. Initially stimulated by a group of less-than-conclusive epidemiological studies,(1) the issue of whether low-level extremely low frequency (ELF) electromagnetic fields can cause observable biological effects has become the focus of a scientific (and political) debate.(2) Beset by replication difficulties, early experiments often confused rather than clarified the situation. This replication problem, which has fueled much of the skepticism about the reality of field-induced bioeffects, can probably now be disregarded. Recently, there have been a number of independent duplications of experimental results. No fewer than four laboratories have detected an ELF-field-induced enhancement of RNA.(3,4,5,6,7,8,9) Several groups have demonstrated increased bone cell proliferation,(10,11,12) while others have detected increased activity of ornithine decarboxylase, an enzyme essential to DNA replication and cell growth, attributable to weak ELF electromagnetic field exposure.(13,14)

There are also cases in which a lack of reproducibility is now understood in terms of subtle differences in the experimental conditions. An example of this is the link between altered chick embryo development and exposure to electromagnetic fields. Interest in this was generated by a 1982 report (15) that exposure to weak 100-Hz pulsed magnetic fields during the first 48 hours of incubation caused an increased rate of abnormal embryo developments. Although the statistical significance of the results was questionable, the work was seminal and inspired numerous repetitions of the experiment. Some of the groups obtained confirmatory data(16,17,18,19) while others were unsuccessful in finding any field-induced abnormal-

ities.(20,21,22) To address this confusion, a six-laboratory collaboration was organized in which each participant carried out the experiment under identical conditions.(23) The result was that two of the six laboratories reported a positive effect while the others saw none (of statistical consequence).

Within the past year, order has emerged from this apparent chaos.(24) The key is recognizing that for electromagnetic fields to induce developmental abnormalities, a genetic predisposition is necessary; moreover, at least in the strain of chick embryos tested, only about 25% have this predisposition. No matter what the exposure conditions, the abnormality rate can not exceed this fraction. When (because of other than electromagnetic stresses) this value is approached in the unexposed control group, there can be *no additional* "field-induced" abnormalities. A strong negative correlation between the rates of field-induced abnormal developments and control-group abnormalities is maintained in all the data sets. In those experiments in which the control group exhibits a high abnormality rate ($> \sim$ 20%), no field-induced abnormalities were reported; when the control-group abnormality rate is small ($< \sim$ 10%), a robust field-enhancement of the abnormality rate was observed. The mystery surrounding the inconsistent observations of field-induced teratogenesis has been unraveled; the data from *eight different laboratories* now form a consistent picture.

Theoretical arguments based on signal-to-noise considerations(25) continue to provoke skepticism however. The dilemma arises because cells, existing in an electrically noisy environment, respond to external EMFs some 100 to 1000 times weaker than local noise fields resulting from the thermaly driven movements of ions in the vicinity of cells. In this context Adair(26) has concluded that "... it does not appear to be possible for weak external ELF electromagnetic fields to affect biological processes significantly at the cell level ..." Yet the data unambiguously demonstrate that they do. How can the externally-impressed fields possibly influence cell behavior when cells have evolved in such a way as to function normally in the presence of the latter? Or phrased another way, how does the cell discriminate against the large thermal noise fields in order to respond to the weak exogenous fields?

As noted in an earlier section, Weaver and Astumian(25) have proposed that cells integrate the electromagnetic signals, effectively narrowing their acceptance bandwidth, and thus averaging out the thermal noise. However, their calculations yield averaging times much longer than exposure intervals observed to produce bioeffects. They estimate that to achieve the required signal-to-noise improvement at 100 Hz requires averaging over 4.3×10^4 s (about 12 h); yet there are many cases of bioeffects being observed with exposure intervals substantially less than one hour. It is clear that a simple time averaging mechanism cannot explain the data.

However, as is also described above, Litovitz, Mullins and Krause (27) have suggested that an important element in the cell's discriminating against thermal noise involves the temporal coherence in the exogenous field. They studied the effect of introducing partial incoherence into the ELF field on the specific activity of ornithine decarboxylase (ODC), a highly inducible enzyme required for DNA replication and cell proliferation. Their studies indicated that four-hour exposure to 100-μT, ELF fields affected the ODC activity (a twofold enhancement) in

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logarithmically-growing murine L929 fibroblasts only if sinusoidal field parameters (such as frequency or amplitude) were held constant for intervals longer than about 10 seconds. No effect was observed in these parameters were varied on a scale less than about 1.0 s; fortimes between 1.0 s and 10 s, an intermediate effect--an increase by a factor between 1 and 2--was found. Essentially the same conclusion has been reached using the chick embryo model system.

It is clear that some type of "signal processing" for about 10 s is essential in the field-induced enhancement of both ODC activity in L929 cells and abnormal development of chick embryos. However, it is also clear that this is not the whole story; ten seconds is not sufficient for detecting a 60-Hz signal that is a factor of 1000 weaker than the ambient thermal noise. Moreover, the field at any point is the sum of the exogenous field and the fluctuating noise field. Since there can be no question that this field exhibits temporal incoherence on a scale much less than 10 seconds, the question remains--how does the cell discriminate against the thermal noise field in order to respond to the exogenous field?

HYPOTHESIS

We propose that living cells discriminate against thermal noise fields by recognizing them as *spatially incoherent*, *i.e.*, uncorrelated at different receptor locations on the cell membrane. We suggest that biolgical cooperativity is an essential feature of cellular response to EMFs; a significant number of receptors at the cell membrane must be simultaneously and coherently activated to produce an effect on the biochemical functioning of the cell. Electromagnetic fields must be spatially coherent if they are to affect cell functioning. This suggests that the exquisite sensitivity to EMFs results from the cells' ability to discriminate against fields which are *both* spatially and temporally incoherent, *i.e.*, against thermal noise.

This idea that a multiplicity of cell signal receptors are simultaneously activated suggests that a mechanism analogous to *coincidence detection* could be operative in discriminating against fields from thermal fluctuations. We assume that the direct "target" of the electromagnetic field is the assembly of neutralizing "counter-ions" in the immediate vicinity of the cell surface, and that the resulting motion of these ions produces effects at the membrane that are transmitted to the cell interior where modification of the biochemical reaction pathway is effected. A plausible supposition is that the ionic motion affects the binding of ligands to the roughly 100,000 receptor proteins (sensors) that are integral to the cell membrane. Binding ligands causes the production of intracellular effector molecules (second messengers) within the cell; the net effect is the transducing of the extracellular signal into an intracellular one. Cooperativity is required in such processes in that "more than one intracellular effector molecule must [simultaneously] bind to some target macromolecule in order to induce a response."(28)

Because the average spacing between receptors is on the order of 100 nm and the Debye screening length (roughly the range over which a given ion is not shielded from other ions) is about 1 nm, localized charge density fluctuations in the neighborhood of a given receptor will not influence motion of charges near other receptor proteins. Thermal noise fields thus are

prevented from producing intracellular effects. Conversely, impressed ELF fields are *spatially* coherent over the cell surface and therefore produce charge density variations that are correlated at various receptor sites in the membrane. Consequently, they produce the required number of effector molecules to initiate a cytoplasmic response. This biological coincidence detection scheme allows the cell to be exquisitely sensitive to very weak *spatially correlated* electromagnetic fields while discriminating against the much stronger but spatially random (on the relevant distance scale) thermal noise fields.

> Therefore, we hypothesize that if a spatially coherent but temporally random noise field were superimposed on the coherent signal, then at some value of the signal/noise amplitude ratio, the discrimination mechanism would be defeated, and the observed field-induced bio-effect would be suppressed.

DEVELOPMENTAL ABNORMALITIES IN CHICK EMBRYOS

In Section II above, a series of studies of EMF-induced avian teratogenesis(24) using pulsed EMFs (100-Hz 500-μs duration 1-μT peak strength) were reported. These offer persuasive evidence (P << 0.01) that EMFs can indeed increase the rate of abnormal development in incubating chick embryos. If a spatially coherent noise field comparable in strength to the impressed EMF--a "confusion" field--were to be also applied, we would expect a reduction in the field-induced abnormalities. We have tested this hypothesis in the same White Leghorn chick embryo model where our previous studies were carried out. The apparatus and techniques followed the "Project Henhouse" protocols (23). In addition to pulsed EMFs, 60-Hz sinusoidal (4 μT peak) and 60-Hz sawtooth wave (6.4 μT peak-to-peak) fields were used. For each case three samples were examined: (1) the sham-exposed (control) sample where no field was imposed; (2) the field-exposed sample; and (3) a sample exposed to a field in which a spatially coherent noise signal was superimposed on the pulsed, sinusoidal, or sawtooth EMF. The noise spectra were flat over a bandwidth of 30 Hz to 100 Hz for the pulsed field and 30 Hz to 90 Hz for the sinusoidal and sawtooth wave fields; the rms noise amplitudes were 1 μT, 4 μT and 6.4 μT, respectively. The results are presented in Table 1.IV.

From the data it is clear that for each waveform, there is an increase in the fraction of abnormally developed embryos when the eggs are subjected to coherent external EMFs. It is also clear that this increase is greatly reduced when a confusion field is present. The statistical significance of these observations was tested by examining the appropriate null hypotheses: (1) the imposition of an EMF has no effect on developing embryos; the difference between the EMF-exposed and control sample results are due to fluctuations in sampling; (2) the superposition of a confusion field on an EMF has no effect on developing embryos; and (3) the abnormality rate for the EMF-plus-noise-exposed and sham-exposed samples do not differ; that is, the confusion field completely masks the effect of the imposed EMF. These hypotheses were tested using a χ^2 test. The P-values (confidence levels) for rejecting these hypotheses are given in Table 2.IV.

Table 1.IV. Results for embryos subjected to square-pulse, sinusoidal and sawtooth EMFs.

Exposure conditions	Live	Abnormal	Percent
100-Hz 1-μT (peak) Pulse EMF			
Sham exposed (control)	255	16	6.3
EMF exposed	152	29	19.1
EMF + noise exposed	120	9	7.5
60-Hz 4-μT (peak) Sinusoidal EMF			
Sham exposed (control)	222	19	8.6
EMF exposed	163	22	13.5
EMF + noise exposed	113	10	8.9
60-Hz 6.4-μT (peak-peak) Sawtooth EMF			
Sham exposed (control)	290	20	6.9
EMF exposed	253	34	13.4
EMF + noise exposed	157	13	8.3

Table 2.IV. Statistical analysis of exposure data from Table 1.IV.

Exposure field	P^a Control- EMF exposed	P^b EMF exposed- Noise + EMF exposed	P^c Control- Noise + EMF exposed
100-Hz 1-μT Pulse EMF	$< 10^{-4}$	0.007	0.65
60-Hz 4-μT (peak) Sine EMF	0.13	0.22	0.85
60-Hz 6.4-μT (p-p) Sawtooth EMF	0.01	0.10	0.50

[a] Probability that the difference in the control and EMF-exposed abnormality rates is due to chance

[b] Probability that the difference in the EMF-exposed and Noise-plus-EMF-exposed abnormality rates is due to chance

[c] Probability that the difference in the control and Noise-plus-EMF-exposed abnormality rates is due to chance

From the results in this table several conclusions can be drawn. (1) Impressing ELF pulsed or sawtooth wave electromagnetic fields with amplitudes on the order of a microtesla induces significant teratogenesis in developing chick embryos (for sinusoidal fields the data are less definitive). (2) Superimposing a confusion field (spatially coherent but temporally incoherent noise) of comparable rms amplitude greatly suppresses the teratogenic effect of these fields. (3) For the noise fields considered here, the abnormality rates with no field imposed and with both the temporally coherent and noise fields are nearly the same. In all cases the abnormality rate with the confusion field is slightly larger than the control sample rate; however, for none of the three waveforms is the difference statistically significant.

ORNITHINE DECARBOXYLASE ACTIVITY IN DEVELOPING CHICK EMBRYOS

In addition to the observation of EMF-induced morphological abnormalities in developing embryos, we have noted in a previous section the modification of the biochemistry involved in embryo development. There we reported a study of the effect on the activity of ornithine decarboxylase (ODC) in White Leghorn embryos subjected to ELF electromagnetic fields.

ODC is a key enzyme in the biochemical pathways which are responsible for polyamine biosynthesis in cells. In the chick embryos, ODC activity has been reported to peak twice within the first 48 hours of development.(29) The first peak is observed at 15 hours of development (gastrulation), and the second at 23 hours (the onset of neurulation and early organogenesis). Our study focused on the 15-h peak of ODC activity corresponding to Hamburger-Hamilton Stage 3. In that work the effect of the EMF (60 Hz, 4 μT amplitude) was to cause a nearly 75% increase in ODC activity.

In the experiment described here, chick embryos were exposed to the sum of (1) a sinusoidal electromagnetic field identical to that used in the first study, and (2) a co-linear temporally incoherent (but spatially coherent) EM "noise" field (4 μT rms, frequency components from 30 to 90 Hz). The ODC activity in embryos exposed to the sum of the sinusoidal and "noise" fields was compared with that in sinusoidal-field-exposed and sham-exposed embryos.

After incubation and exposure, the embryos were separated according to the standard Hamburger and Hamilton stages. In order to eliminate unintentional variability due to possible incubation before arrival at the lab, only embryos of the proper stage (H&H stage 3) were retained for analysis. A scalpel was then used to trim the area opaqua from the blastoderm so that only the area pellucida remained. This insured that only the embryo area proper was analyzed. The embryos were pooled according to incubator, with 4 to 6 embryos per tube, centrifuged, and frozen. A total of 60 embryos were used for the study. The assay for ODC is standard and is described above in Section I.

The results (Table 3.IV) support the hypothesis that a superimposed EM noise field masks the sinusoidal field, thus blocking its bioeffects. For stage 3 embryos, the ODC activity

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of sinusoidal field-exposed embryos was increased by field exposure, consistent with the result reported earlier. On the other hand, the ratio of activity in embryos exposed to the sum of the sinusoidal and noise fields to the controls was nearly unity. In other words, the ODC activity in embryos exposed to the sum of the coherent and noise fields was virtually indistinguishable from that in the control embryos. The modification of embryo biochemistry induced by a weak sinusoidal field can be blocked by the superposition of a spatially coherent, temporally incoherent EM field of comparable strength.

Table 3.IV: ODC activity in embryos exposed to sinusoid-plus-noise field

Stage	Number of Embryos	ODC Activity	
		Exposed/Control (Sine)*	Exposed/Control (Sine + Noise)
3	60	1.98 ± 0.57	0.98 ± 0.06

* The ODC activity ratio for the embryos exposed to sinusoidal fields were computed only for the replicates in which sinusoid-plus-noise fields were also imposed. Thus, the exact numbers differ from those in Section 2 which includes all experiments in which embryos were exposed to sinusoidal fields.

These findings support our proposal that it is their sensitivity only to *spatially coherent* EMFs that enables living cells to respond to weak exogenous fields while remaining unaffected by the relatively large (but spatially incoherent) endogenous noise fields that are always present. The idea that cells distinguish between exogenous and endogenous fields by recognizing the latter's spatial incoherence offers a viable explanation of the signal-to-noise dilemma. Far from being "magically" exempt from signal-to-noise considerations, cellular detection mechanisms are subject to the same laws of nature as all physical systems. The masking that is provided by a spatially coherent confusion field could prove to be a basis for protecting humans against possible adverse health risks associated with environmental electromagnetic fields.

In summary, we have presented a hypothesis that is consistent with the extant data that accounts for the sensitivity of cells to external electromagnetic fields that are several orders of magnitude weaker than endogenous thermally-driven noise fields. The idea is that cellular response to a field requires simultaneous activation of several membrane sensors, thereby enabling cells to discriminate against spatially incoherent thermal noise while maintaining sensitivity to correlated external signals. When a temporally random, but spatially correlated noise signal is combined with a sinusoidal field, the cell's coincidence-detection discrimination mechanism becomes inoperative, it faces the usual signal-to-noise problems, and the biological effect is suppressed.

REFERENCES

1.   See, for example N. Wertheimer and E. Leeper, Am. J. Epidemiol. 109, 273 (1979); 111, 461 (1980); 129, 220 (1989); S. Milham, Jr., New Engl. J. Med. 307, 249 (1982); Occupational mortality in Washington State, 1950-1979, DHEW NIOSH Publication No. 83-16 (1983); Env. Health Perspect. 62, 297 (1985); D. A. Savitz, Childhood cancer and electromagnetic field exposure (New York State Department of Health, Power Lines Project, Albany, NY, 1987); D. A Savitz et al., Am. J. Epidemiol. 128, 21 (1988).

2.   I. Nair, M. G. Morgan and H. K. Florig, Biological Effects of Power Frequency Electric and Magnetic Fields--Background Paper, U. S. Congress, Office of Technological Assessment Report No. OTA-BP-E-53 (US Government Printing Office, Washington, DC, 1989).

3.   R. Goodman, C. A. L. Bassett and A. S. Henderson, Science 220, 1283 (1983).

4.   R. Goodman and A. S. Henderson, Bioelectromagnetics 7, 23 (1986); Proc. Nat. Acad. Sci. USA 85, 3928 (1988).

5.   R. Goodman, J. Abbott and A. S. Henderson, Bioelectromagnetics 8, 1 (1987).

6.   R. Goodman, L-X. Wei, J-C. Xu and A. S. Henderson, Biochem. et Biophys. Acta 1009, 216 (1989).

7.   E. Czerska et al., Comparison of the effect of ELF on C-MYC oncogene expression in normal and transformed human cells, contribution to Thirteenth Annual Meeting of the Bioelectromagnetics Society, Salt Lake City, June 23-27, 1991, unpublished.

8.   J. M. Mullins, J. J. Greene and D. Krause, private communication.

9.   J. L. Phillips, W. Haggren and W. R. Adey, Effects of 60-Hz magnetic field exposure on gene transcription in T-Lymphoblastoid cells, contribution to Annual Review of Research on Biological Effects of 50- and 60-Hz Electric and Magnetic Fields, Denver, CO (November 5-8, 1990), unpublished.

10.   C. T. Brighton, E. Okereke, S. R. Pollock and C.C. Clark, J. Orth. Res. (to be published).

11.   C. T. Rubin, K. J. McLeod and L. E. Lanyon, J. Bone Joint Surg. 71-A, 411 (1989).

12.   D. Ciomber, R. Aarons and C. Polk, private communication.

13.   D. Krause, et al., Biochem. Biophys. Res. Comm. (to be published).

14.   C. V. Byus et al., Carcinogenesis 6, 859 (1987); Cancer Res. 48, 4222 (1988).

15.   J. M. Delgado et al., J. Anat. 134, 533 (1982).

16.   A. Ubeda et al., J. Anat. 137, 513 (1983).

17.   J. Juutilainen et al., Radiat Environ. Biophys. 25, 65 (1986); 25, 135 (1986).

18.   J. Juutilainen, E. Läära and K. Saali, Int. J. Radiat. Biol. 52, 787 (1987).

19.   A. H. Martin, Bioelectromagnetics 9, 393 (1988); Electromagnetic fields and the developing embryo, contribution to Annual Review of Research on Biological Effects of 50- and 60-Hz Electric and Magnetic Fields, Portland, OR (November 13-16, 1989), unpublished; ELF, PEMF's and development: why the controversy? Proceedings of the XXIII General Assembly of URSI, Prague Czechoslovakia, Aug. 28-Sept. 5, 1990 (to be published).

20.   S. Maffeo et al., J. Anat. 139, 613 (1984); 157, 101 (1988).

21.   B. F. Sisken, J. Bioelec. 5, 25 (1986).

22.   M. Sandström, K. H. Mild and S. Løvtrup, in Work with Display Units, B. Knave and P-G. Wideback, eds. (Elsevier Science Publishers, Amsterdam, 1986), pp. 135ff.

23.   E. Berman, L. Chacon, D. House. B. A. Koch, W. E. Koch, J. Leal, S. Løvtrup, E. Mantiply, A. H. Martin, G. I. Martucci, K. H. Mild, J. C. Monahan, M. Sandström, K. Shamsaifar, R. Tell R, M. A. Trillo, A. Ubeda, and P. Wagner P, The effects of a pulsed magnetic field on chick embryos, Bioelectromagnetics 11, 169-187 (1990).

24.   T. A. Litovitz, C. J. Montrose, P. Doinov, M. Barber. K. Brown and T. L. Litovitz, Early developmental abnormalities in chick embryos exposed to pulsed electromagnetic fields (submitted for publication).

25.   J. C. Weaver and R. D. Astumian RD, The response of living cells to very weak electric fields: the thermal noise limit, Science 247, 459-462 (1990).

26.   R. K. Adair, Constraints on biological effects of weak extremely-low-frequency electromagnetic fields, Phys. Rev. A43, 1039-1048 (1991).

27.   D. Krause, J. M. Mullins and T. A. Litovitz, Biochem. Biophys. Res. Comm. (submitted for publication).

28.   B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts and J. D. Watson, Molecular Biology of the Cell (Garland Publishing, Inc., New York, 1983) p. 752.

29.   B. Lowkvist, O. Heby and H. Emmanuelsson, Essential Role of the Polyamines in Early Chick Embryo Development, J. Emb. Exp. Morph. 60, 83-92 (1980).

V. Electronic Instrumentation for Experimental Studies

The instrumentation that has been developed and presently in use in our laboratories has provided for a wide range of electromagnetic exposure experiments. The instrumentation combines commercially available equipment and special purpose components which, where necessary were designed and developed at Catholic University.

The instrumentation broadly categorized are:

a. Microwave Systems

These particular facilities were developed and provided to the Biology group for the in-vitro studies. The early measurements were concerned with cultured cell samples in suspension irradiated with continuous microwave power at signal frequency of 2.45 GHz. A Thermostated waveguide section was designed to form a sample holder which could be operated in the CW or pulse modes. The specific absorption rates (SAR) encountered in these studies were in the range from 0.05 to 1200 mW/grm. In addition to the Continuous Wave (CW) measurements, a series of microwave pulse measurements were made. The average power and consequently the SAR for these pulse studies was maintained at the CW levels given above by irradiating with microsecond pulses at 1 and 10 microseconds having appropriate low repetition rates. A waveguide section called "the applicator" developed
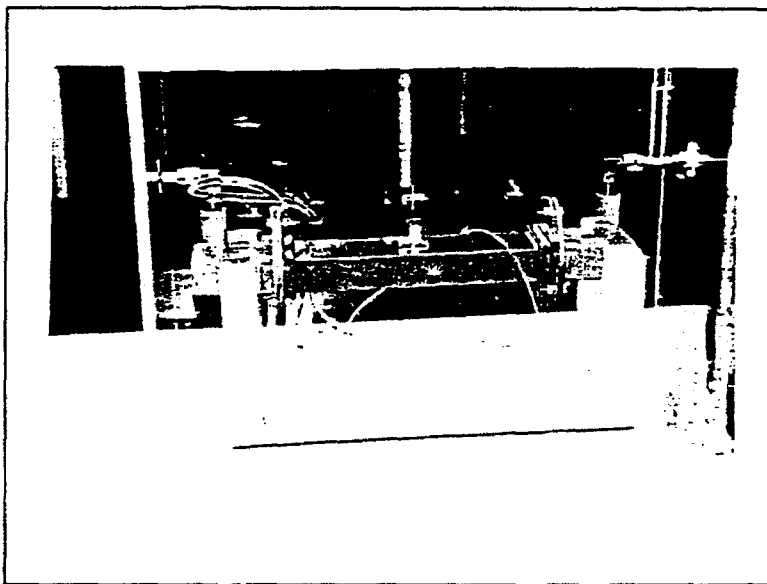

Figure 1.V. Photo of the Waveguide Applicator.

to irradiate the cell sample is shown in Figure 1.V. The applicator is constructed from a section of S-Band rectangular waveguide WR-284 within which is installed a temperature controlled bath. The bath consists of a plexiglass coaxial cylindrical container through which liquid freon is circulated. The cultured cells sample under study is suspended in a nutrient and contained in a test tube which is inserted at the top of the guide as shown in the Figure 2.V and housed within the temperature bath. The block diagram of the instrumentation is shown in Figure 3.V. A Hewlett Packard microwave

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sweep generator (1) which provides an input to a 200 watt travelling wave amplifier TWT (2) provides power to irradiate the cultured cell samples. The system can provide continuous wave (CW), sine or pulse wave modulation at 2.45 GHz carrier. Instrumentation for the microwave power measurements and are shown in the block diagram Fig. 3.V. The temperature was maintained @ 37 C with the use of a Lauda K-1/R refrigeration system to cool the freon bath (Fig. 2.V). The system described above was used primarily to study cell cultures in suspension.

Following these microwave waveguide in-suspension studies a second microwave irradiation facility was developed to allow exposure study of cultured cells in a monolayer configuration. The microwave irradiation applicator used in this series of measurements was in the form of a stripline transmission system. This microwave applicator called the "Crawford Cell" which is commercially available(3) and shown in Figure 4.V provides a picture of this instrument. The Crawford cell was housed within a temperature controlled incubator. The system can operate between 0.8 and 1.0 GHz. The system operates at a signal frequency of 0.915 GHZ. A

Figure 2.V. Thermostatic bath for temperature control of test tube sample holder.

maximum incident power of 10 Watts is available with the aid of a traveling wave amplifier TWT(4). The signal generator(1) driving the TWT can be operated in continuous wave (CW) mode, and also with sine or pulse modulation.
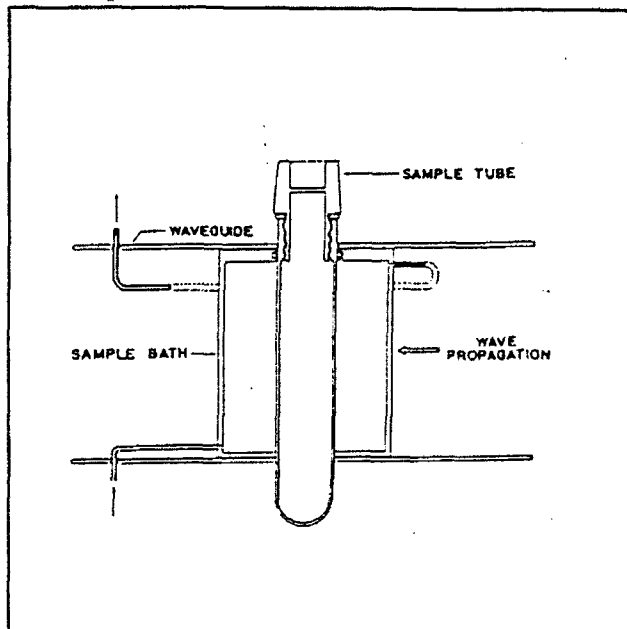
The Crawford cell supported in a verticle position was modified to accommodate shelves positioned parallel to the electric field at either side of the center conductor for cell-sample insertion positioning. The Crawford cell mounted vertically on a rotating stand was placed in an incubator for ease of loading. Rotary coaxial joints were connected at both input and output ports of the Crawford cell to allow free rotation which permitted access for sample insertion from the front of the incubator. Sample containers were placed symmetrically at either side of the center conductor to maintain symmetry of the electric field within the Crawford cell. TEM propagation was assumed in estimating the electric field strength propagating along this strip line configuration.

Measurements were made using 25 cm² tissue culture flasks. The flasks were used, two on each side of the center conductor of the Crawford cell Figure 4.V. The effective specific absorption rate SAR at various power levels was determined by making
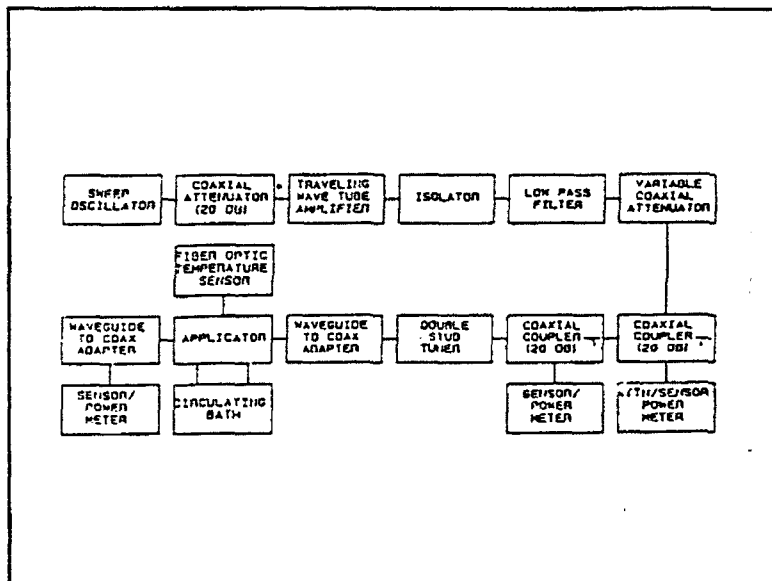
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Figure 3V. Block diagram of microwave Waveguide Applicator and associated instrumentation.

measurements of the incident, reflected, and transmitted power to compute the net power loss and SAR.

In the course of these microwave radiation studies it was determined that the continuous wave radiation of fixed amplitude did not result in a bioeffect. Reported microwave irradiation with modulated carrier at extremely low frequency ELF, produced positive and reproducible effects (5). Measurements in our lab were made with sine wave amplitude modulation at 6, 16, 55, 60, 65 and 600 Hz. The microwave (CW) carrier was amplitude modulated at 23% modulation.

The question arose as to whether periodically changing the modulated microwave signal during the course of a measurement would interfere with bioeffect. Instrumentation was assembled which would provide such flexibility to study these effects. Since 60 Hz modulation

Figure 4.V - Photo of Crawford Cell.

introduced a positive bioeffect during irradiation it was decided to apply a signal which maintained the 23% modulation but shifted the frequency of modulation discretely from 55 to 65 Hz. In this arrangement the time sequencing of the shift between the two separate frequencies was programmed with a D/A interface to be on for a duration with period T minus a random interval 0-0.055 sec. The shifting of frequency was accomplished via amplitude input to a voltage to frequency (V/F) converter (6). The duration T was adjusted in order to determine whether there was an approximate minimum time for the positive biological effect. Subsequently the random portion of the interval was eliminated. The various periods T included one tenth, one, five, ten, fifty seconds. From the measurements which have been discussed earlier in this final report

(Section I) a minimum of ten seconds exposure was found to be required for full biological effect. Irradiation periods in the neighborhood of one to five seconds produced intermediate results.

b.   Extremely Low Frequency ELF Systems.

The major factors that encouraged expansion of our studies to the low frequency range ELF in the neighborhood of 60 Hz mainly resulted from the works of Byus et al (7). The work of Byus included both the amplitude modulated microwaves as indicated in part a. as well as ELF frequency studies. Several Cell lines studied by Byus provided positive bioeffect using ornithine decarboxylase (ODC) as a marker. The ELF exposure works of Goodman et al (8) on the human leukemia line HL-60 supported increased messenger RNA gene expression. In addition to these invitro studies, irradiation studies on fertilized chick eggs (9) also had yielded interesting effects produced by ELF exposure. In the studies called "Project Henhouse" skeletal deformities occurred with ELF exposure during early development stages (Section II).

The ELF instrumentation for the in-vitro studies which was assembled provided magnetic flux densities from approximately one microtesla to one thousand microtesla (10 miligauss to 10 gauss.) The signal frequency chosen for most of these C.W. measurements was 60 Hz. The block diagram figure
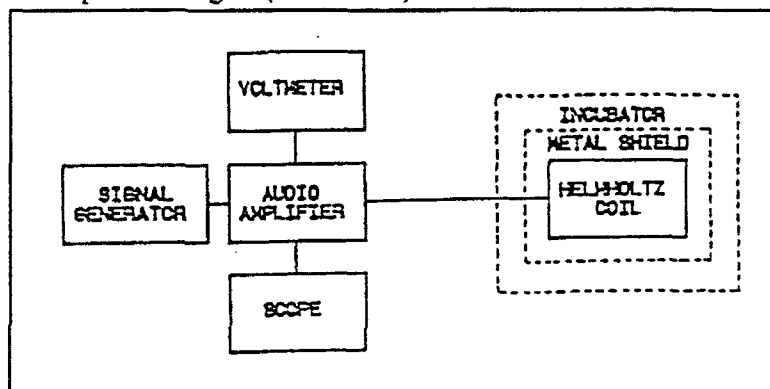

Figure 5.V.   Basic instrumentation for excitation of ELF systems.

5.V provides the essential features of the system used. The physical coil configurations to provide the desired magnetic field were of the Solenoidal and the Helmholtz type. A brief discussion of each is given to illustrate the advantage accrued. All measurements were made with subconfluent monolayer of cultured cells which adhered to the bottom of a petri dish or flask.

Solenoidal Exposure System, In-Vitro Measurement.

A question of fundamental importance in these studies is concerned with the relative roles of the electric and magnetic field to which the cultured cell is exposed.

In setting up the solenoidal configuration figure 6.V it became apparent that while the magnetic field was uniform within the cylinder, the placement of dishes containing the cultured cell samples arranged orthogonal to the cylindrical axis experienced an electric field which increased with radius within the circular petri dish. Such an
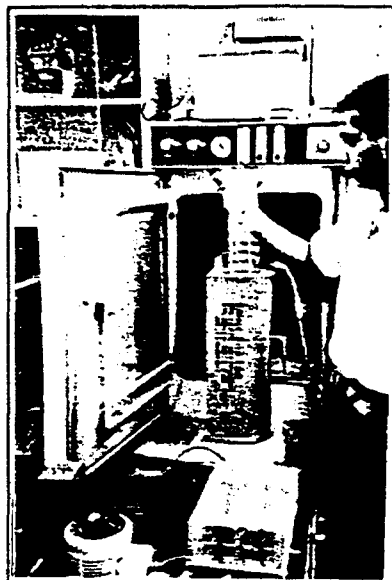
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Figure 6.V Photo of the Solenoid Coil showing insertion of sample dishes.

arrangement allowed a method for distinguishing between the electric and the magnetic field effect. Because of the simplicity of geometry resulting from the orientation of petri dish within the solenoid figure 7.V, the application of Faraday's Law allows direct determination of the electric field from the simple formula. By using dishes with coaxially cylindrical and separate compartments of increasing radius, figure 8.V, sets of samples were simultaneously exposed to different electric fields. Measurements of electric field were presented in a paper Bassen et.al (10) as well as providing quantative methods for determining fields for containers of several geometries and orientations with respect to the applied magnetic field. The abstract provides the rationale and essential features of this study: "Many in-vitro experiments on the biological effects of extremely low frequency (ELF) electromagnetic fields utilize a uniform external magnetic flux density strength (B) to expose biological materials. A significant number of researchers do not measure or estimate the resulting electric field strength or current density that is induced in the sample in the sample medium. The magnitude and spatial distribution of the induced E field are highly dependent on the sample geometry and its relative orientation with respect to the magnetic field. Studies have been made of the electric fields induced in several of the most frequently used laboratory culture dishes and flasks under various exposure conditions. Measurements and calculations of the electric field distributions in the aqueous sample volume in the containers were performed, and a set of simple, quantitative tables are developed. These tables allow a researcher to determine, in a straightforward fashion, the magnitudes and distributions of the electric fields that are induced in the aqueous sample when it is subjected to a uniform, sinusoidal magnetic field of known strength and frequency. In addition, a novel exposure technique is presented using a standard organ culture dish containing two circular, concoentric annular rings. Exposure of the organ culture dish with a uniform magnetic field induces different average electric fields in the liquid medium in the inner and outer rings. Results of experiments using this system, have shown the dominant role of the magnetically-induced electric field in producing specific biological effects on cells, in-vitro. These results emphasize the need to report data about the induced electric field in ELF in-vitro studies using magnetic field exposures. Data tables on electric field and current density in standard containers provide simple means to enable determination of these parameters."
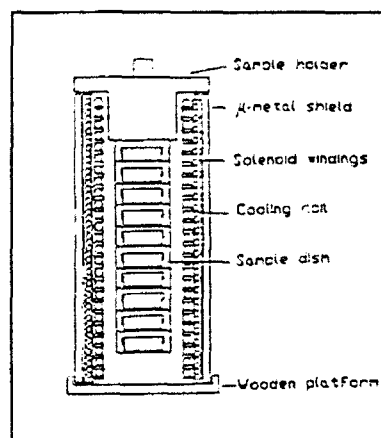

Figure 7.V. Axial cross-section of solenoid showing plexiglass sample holders.

The resulting experimental study of J. Green (11) on leukemia HL60 cells provides direct evidence that the magnitude of the electric field is the dominant cause of the detected bioeffect. As discussed experimental apparatus design allows comparison of the effect of different magnitudes of magnetic field with a constant electric field or constant magnetic field with different electric field.
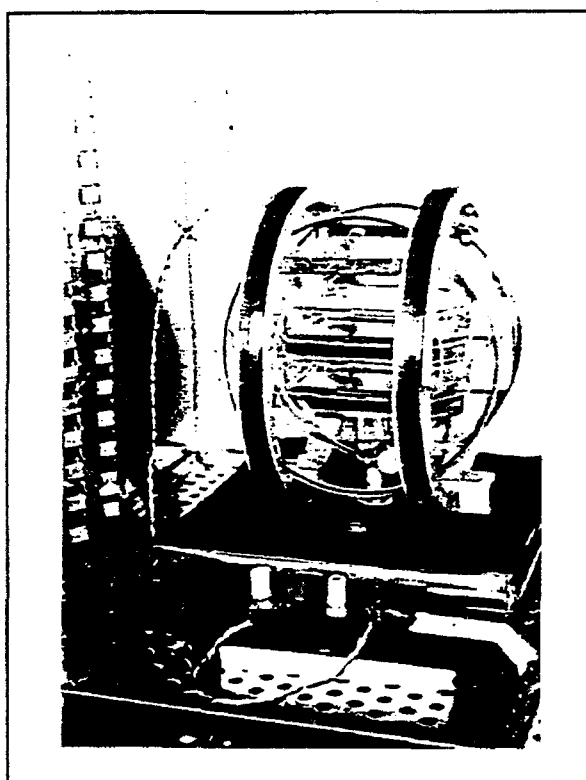

Figure 8.V. A Photo of dishes with coaxially cylindrical separate compartments.

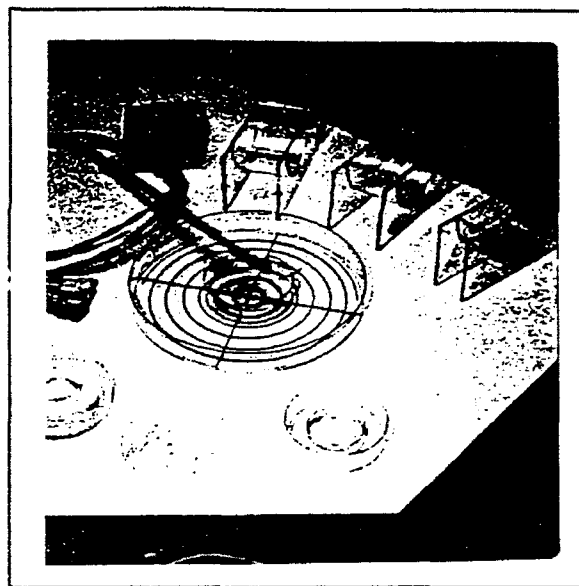Figure 9.V. Photo of Helmholtz coil configuration showing an orthogonal set of coils and the sample holders.

Helmholtz Exposure System,
In-Vitro Measurements

The solenoidal ELF system just described provided a simple method of exposing the monolayer of cells to a magnetic field which is perpendicular to the layer of the biocells.

A method which has been used extensively for these low frequency studies makes use of the Helmoltz coil configuration, Figure 9.V. The cell monolayer cultures are also used in these studies. The physical structure of the Helmholtz coil allows orientation of the magnetic field either perpendicular or parallel to the monolayer. In all of the in vitro cell studies presented in this work using the Helmholtz the parallel orientation was used.

The determination of the magnitude of the electric field in this orientation is much more complicated than for the solenoidal case (12,10). The electric field in the solenoidal case considering circular geometry of the dish and that cells and nutrient are homogeneous (even though the monolayer is subconfluent) and resulted in simple expression for the electric field. However in the case of the parallel magnetic field the electric field forming a closed loop passed through the monolayer enters a layer of nutrient and returns to the monolayer. The physical arrangement resembles a bilayer and must be treated in this manner to determine the electric field in the monolayer. In general the two layers would have electrical parameters including dielectric constant and conductivity which are different. In general the electric fields in the different regions induced by the uniform magnetic field would differ. A solution to this boundary problem showed that the fields in both regions for the case of a monolayer (a very thin layer) are the same. Measurements confirmed these results. The results of this study were presented in a paper oy Wang et.al.(13). The abstract provides the essential features of this study: "In-vitro studies of biological effects of electromagnetic fields are often conducted with the biological cultured cells either in suspension or grown in a monolayer. In the former case, the exposed medium can be assumed to be homogeneous; however, the cells eventually settle to the bottom of the contained forming a two layer system with different dielectric and conductive properties. Since a portion of our measurements are made in the monolayer configuration it is important to determine electric field near the bottom of the container where separation occurs, namely the nutrient above the cell monolayer. In the present work, the effect of this separation on the electric field distributions calculated and experimentally measured at selected positions for a commonly used exposure configuration. The settled cell suspension is modeled using a well defined two layer system placed in a rectangular container with the base of the container parallel to the direction of the magnetic field. Theoretical calculations using numerical techniques are carried out for various two layer systems with conductivity of each layer different. When the thickness of one of the layers is small compared to the thickness of the other layer, i.e., the bottom layer of cells, it is found that the electric field distribution is essentially that of the homogeneous case. The situation corresponds to a typical call exposure condition."

The electric fields that have been discussed are fields that are assumed to pass through this homogeneous medium. A comment on the electric field that may exist within the living cell is appropriate While electric field measurements within a given cell could not be made, a series of experimental measurements were made in a system which might model the cell in the nutrient. The experiment was set up using three small circular Petri dishes of different diameters placed within a large circular Petri dish. All dishes were filled with a conducting liquid. An electric field probe of small dimensions was fabricated. The measurements that were made clearly demonstrated that a time varying magnetic field normally incident on the base of the system of dishes described that the magnitude of the electric field in each container is determined independently of its neighbors. The magnitude of the field with each circular container (dish) increases with radius from the center of the dish in similar fashion to that described in the solenoidal experiment discussed earlier. Carrying these results to the biocell would

indicate that the electric field within the cell, because of its small diameter, about 10 μcrons, is extremely small (of the order of 300 pV/cm). The external electric field on the cell membrane is significantly higher than this value.

Helmholtz Exposure System, In-Vivo Measurements

Extensive measurements have been reported about effect of pulsed magnetic fields on development of Chicken Embryos. A coordinated effort using identical exposure systems and shown in figure 10.V was launched in six laboratories and distributed in several countries including the United States. The study was made to accumulate data from independent laboratories to answer critical questions (9). One of these systems was obtained from the Food and Drug Administration to make the same measurements using identical protocol and to study the details of measurement procedure. In addition to the original protocol for the pulse studies, measurements in this lab were also made using 60 Hz sinusoidal waves and 60 Hz saw tooth waves to drive the Helmholtz coils as described in Section II. Six systems were installed for efficient data and these results are reported in section II. In addition, a modification of two Helmholtz systems included a second set of Helmholtz coils which could be oriented coaxially or othogonally to the original coils for simultaneous application of different signals.
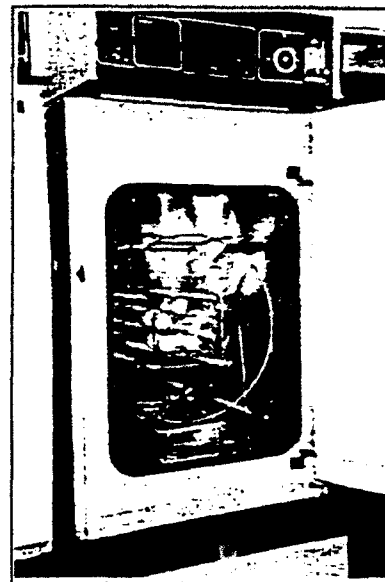

Figure 10.V. Helmholtz Coils loaded with eggs in incubator. Temperature controlled at 37.8°.

c.   Coherence

The ELF experimental system so far discussed in this section V involved sine wave and pulse irradiation radiation. Extensive measurements were also reported in sections I, III, V, which included effects of band limited noise. For the in-vitro studies where band limited noise was to be simultaneously applied to the cultured cell samples the instrument configuration included a noise generator, whose amplitude could be varied, and an adjustable filter. The noise was mixed directly with the 60 Hz sine waves in a mixing amplifier. In this way effects of the application of the noise could be determined (section Ie). For the in-vivo Chicken Embryo studies band limited noise was applied to one of the orthogonal set of coils while applying the "original protocol" pulse train to the other set of coils. The results of these studies are discussed in Section IV.

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REFERENCES

1. Hewlett Packard: HP 8350B Sweep Oscillator/HP 83540A RF Plug-in 2.0 to 8.4 GHz.

2. Varian Associates: Travelling Wave Amplifier Model VZS693G51125 200 watt 2-4 Ghz.

3. Instruments for Industry, Inc.: Crawford Cell Test Chamber Model CC110 DC - 1000Mhz.

4. Amplifier Research Corp.: Travelling Wave Amplifier Model 10W1000, 10 Watts

5. Craig V. Byus, K. Kartum, S. Pieper, and W. Ross Adey, August 1, 1988, Increased Ornithine Decarboxylase Activity in Cultured Cells Exposed to Low Energy Modulated Microwave Fields and Phorbol Ester Turmor Promoters. Cancer Research 48, 422-4226.

6. TENMA: Function Generator Model 72-380.

7. Craig V. Byus, Susan E. Pieper and W. Ross Adey, 1987, The effects of low-energy 60-Hz environmental electromagnetic fields upon the growth-related enzyme ornithine decarboxylase, Carcinogenesais vol 8. no. 10 pp 1385-1389.

8. Reba Goodman and Ann S. Henderson, June 1988, Exposure of salivary gland cells to low-frequency electromagnetic fields alters polypeptide synthesis, Proc. Natl. Acad. Sci. USA, vol. 85, pp. 3928-3932, Genetics.

9. E. Berman, et.al, 1990, Development of Chicken Embryos in a Pulsed Magnetic Field, Bioelectromagnetics 11:169-187.

10. H. Bassen, T. Litovitz, M. Penafiel, and R. Meister, 1992, ELF In Vitro Exposure Systems for Inducing Uniform Electric and Magnetic Fields in Cell Culture Media, Bioelectromagnetics 13:183-198.

11. James J. Greene, et.al., 1991, Delineation of Electric and Magnetic Field Effects of Extremely Low Frequency Electromagnetic Radiation on Transcription, Biochemical & Biophysical Research Communication, vol 174. pp 742-749.

12. Bruce R. McLeod, Arthur A. Pilla and Michael W. Sampsel, 1983, Electromagnetic Fields Induced by Helmhnoltz Aiding Coils Inside Saline-Filled Boundaries, Bioelectromagnetics 4:357-370.

13. W. Wang, T. Litovitz, M. Penafiel, R. Meister, Determination of the Induced E.L.F. Electric Field Distribution in a Two-Layer In-Vitro System Simuilating Biological Cells in Nutrient, to be published Bioelectromagnetic, Fall 1992.

14. Food & Drug Administration: Bureau of Radiological Health.

APPENDIX

PERSONNEL RECEIVING SUPPORT DURING
THE PERIOD OF THIS STUDY
1987-1992

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THE CATHOLIC UNIVERSITY OF AMERICA
BIOELECTROMAGNETISM RESEARCH GROUP

PAPERS PUBLISHED OR SUBMITTED FOR PUBLICATION

1. T.A. Litovitz, C.J. Montrose, Reba Goodman and Edward C. Elson, *Amplitude Windows and transiently augmented transcription from exposure to electromagnetic fields*, Bioelectromagnetics 11:297-312 (1990).

2. C.J. Montrose, T.A. Litovitz and E.C. Elson, *Augmented transcription by electromagnetic field exposure: the experimental technique affects the measured transient response*, Bioelectrochemistry and Bioenergetics, 25:383-394 (1991).

3. B. Saif, R.K. Mohr, C.J. Montrose and T.A. Litovitz, *On the mechanism of dielectric relaxation in aqueous DNA solutions*, Biopolymers (scheduled for publication).

4. T.A. Litovitz, C.J. Montrose and T.L. Litovitz, *On the adverse effects of electromagnetic fields*, Proceedings of the International Conference on Environmental and Industrial Toxicology: Research and Applications, Bangkok, Thailand, July 22-26, 1991 (scheduled for publication).

5. T.A. Litovitz, C.J. Montrose and W. Wang, *Dose response implications of the transient nature of electromagnetic-field-induced bioeffects: theoretical hypotheses and predictions*, Bioelectromagnetics (scheduled for publication, 1992).

6. T.A. Litovitz, D. Krause and J.M. Mullins, *Effect of coherence time of the applied magnetic field on ornithine decarboxylase activity*, Biochem. Biophys. Res. Comm. 178:862-865 (1991).

7. H. Bassen, T.A. Litovitz, L.M. Penafiel and R. Meister, *Recommendations for standardization of sample containers used for in-vitro ELF biological exposure systems*, Bioelectromagnetics 13:183-198 (1992).

8. W. Wang, T.A. Litovitz, L.M. Penafiel and R. Meister, *Determination of the induced electric field distribution in a two-layer in-vitro system simulating biological exposure systems*, Bioelectromagnetics (scheduled for publication, 1992).

9. L.M. Penafiel and T.A. Litovitz, *Counter-ion condensation and ionic conductivity*, J. Chem. Phys. 96:3033-3038 (1992).

10. L.M. Penafiel and T.A. Litovitz, *High frequency dielectric dispersion of polyelectrolyte solutions and its relation to counter-ion condensation*, J. Chem. Phys. 97:559-567 (1992).

11. C.J. Montrose and T.A. Litovitz, *Biological response to weak ELF electromagnetic*

fields, Physics and Society 21:7-8 (1992).

12.   T.A. Litovitz, C.J. Montrose and P. Doinov, *Spatial and temporal coherence affects the response of biological systems to electromagnetic fields*, Proceedings of the First World Congress on Bioelectromagnetism, Orlando, Florida, June 14-18, 1992 (to be published).

13.   J.M. Mullins, D. Krause and T.A. Litovitz, *Simultaneous application of a spatially coherent noise field blocks the response of cell cultures to a 60-Hz electromagnetic field*, Proceedings of the First World Congress on Bioelectromagnetism, Orlando Florida, June 14-18, 1992 (to be published).

14.   J.M. Farrell, M. Barber, P. Doinov, D. Krause and T.A. Litovitz, *Effect of 60-Hz sinusoidal magnetic fields on ornithine ecarboxylase activity in developing chick embryos*, Proceedings of the First World Congress on Bioelectromagnetism, Orlando, Florida, June 14-18, 1992 (to be published).

15.   T.A. Litovitz, C.J. Montrose, P. Doinov, M. Barber, K. Brown and T.L. Litovitz) *Avian teratogenesis induced by pulsed electromagnetic fields*, Bioelectromagnetics (submitted for publication).

16.   J.J. Greene, W.J. Skowronski, J.M. Mullins, R.M. Nardone, M. Penafiel and R. Meister, *Delineation of electric and magnetic field of extremely low frequency electromagnetic radiation on transcription*, Biochem. Biophys. Res. Comm., 174:742-749, 1991.

17.   D.J. Krause, J.M. Mullins, L.M. Penafiel, R. Meister and R.M. Nardone, *Microwave exposure alters the expression of 2-5A-Dependent RNase*, Radiation Res., 127:164-170, 1991.

18.   D. Krause, W.J. Skowronski, J.M. Mullins, R.M. Nardone and J.J. Greene, *Selective enhancement of gene expression by 60 Hz electromagnetic radiation*, Electromagnetics in Biology and Medicine (C.T. Brighton and S.R. Pollack, Eds.) pp. 133-138, San Francisco Press, Inc., San Francisco, 1991.

19.   D.J. Krause, J.M. Mullins, L.M. Penafiel, R. Meister and R.M. Nardone, *Alteration of ornithine decarboxylase activity by amplitude-modulated, but not continuous wave microwave exposure*, Radiation Res., Submitted.

20.   T., Litovitz, D.J. Krause, and J.M. Mullins, *The role of coherence time in the effect of microwaves on ornithine decarboxylase activity*, Bioelectromagnetics, submitted, 1992.

21.   J.M. Mullins, T. Litovitz and D. Krause, *Simultaneous application of a spatially coherent noise field blocks the response of cell cultures to a 60 Hz electromagnetic field.* Proceedings, First World Congress for electricity and Magnetism in Biology and Medicine, 1992.

22. J.J. Greene, S.L. Pearson, W.J. Skowronski, R.M. Nardone, J.M. Mullins and D. Krause, *Gene-specific modulation of RNA synthesis and degradation by extremely low frequency electromagnetic fields*, Archives of Biochem. and Biophys. submitted.

23. T.A. Litovitz, J.M. Mullins, D. Krause, *The role of coherence time in the effect of microwaves on ornithine decarboxylase activity*, Biochem. Biophys. Res. Comm., **178**:862-865, 1992.

24. D. Krause, L.M. Penafiel, R. Myers, R. Meister, R.M. Nardone and J.M. Mullins, *Enhancement of ornithine decarboxylase activity in L929 cells by amplitude module microwaves*, Radiation Research, 1992.

25. J.M. Mullins, D. Krause and T.A. Litovitz, *Spatial coherent noise blocks enhancement of ODC activity by 60 Hz ELF*, in preparation.

PAPERS PRESENTED AT MEETINGS

1. D. Krause, L.M. Penafiel, J.A. Brent, L.A. Shonk, J.M. Mullins and R.M. Nardone, *Effect of 2.45 GHz microwaves on two interferon-regulated enzymes*, presented at the 10th annual meeting The Bioelectromagnetics Society, Stamford, CT, June, 1988.

2. T.A. Litovitz, C.J. Montrose, Reba Goodman and Edward C. Elson, *A mathematical model for the effect on cellular transcription of low frequency electromagnetic radiation*, presented at the 11th annual meeting of The Bioelectromagnetics Society, Tucson, AX, June 18-22, 1989.

3. D. Krause, J.A. Brent, J.M. Mullins, J.J. Greene and R.M. Nardone, *Induction of c-myc and histone H2B in HL-60 cells by extremely low frequency (ELF) electromagnetic radiation (EMR)*, presented at the 11th annual meeting of The Bioelectromagnetics Society, Tucson, AZ, June 18-22, 1989.

4. H. Bassen, T.A. Litovitz and R. Meister, *Improved dosimetry is needed for ELF in vitro research*, presented at the 11th annual meeting of The Bioelectromagnetics Society, Tucson, AZ, June 18-22, 1989.

5. J.J. Greene, W.J. Skowronski, D. Krause, J.M. Mullins and R.M. Nardone, *Delineation of the magnetic and electric field contributions to the enhancement of transcription by low frequency radiation*, presented at the 11th annual meeting of The Bioelectromagnetics Society, Tucson, AZ, June 18-22, 1989.

6. D. Krause*, J.M. Mullins, L.M. Penafiel, J.J. Greene, W.J. Skowronski, D.R. Davis, J.A. Brent, C.J. Montrose, R.M. Nardone, R. Meister, and T.A. Litovitz, *Effects of microwave and ELF electromagnetic fields on cells in culture*, invited presentation at the Federal Drug Administration, Rockville, MD, October 1989.

85

7. D. Krause, W.J. Skowronski, J.M. Mullins, R.M. Nardone, and J.J. Greene, *Magnetic and electric field contributions to bioeffects during in vitro studies using ELF magnetic fields*, invited presentation at the Battelle Pacific Northwest Laboratories/National Institute of Standards and Technology Workshop, San Antonio, TX, June 9, 1990.

8. C.J. Montrose and T.A. Litovitz, *Understanding frequency and power windows with a dynamic multistep reaction model*, presented at the 12th annual meeting of The Bioelectromagnetics Society, San Antonio, TX June 10-14, 1990.

9. B. Saif, T.A. Litovitz, C.J. Montrose and R.K. Mohr, *On the origin of microwave absorption in DNA solutions*, presented at the 12th annual meeting of The Bioelectromagnetics Society, San Antonio, TX, June 10-14, 1990.

10. L.M. Penafiel, R. Meister and T.A. Litovitz, *Localized microwave absorption in DNA solutions is revealed by the static conductivity*, presented at the 12th annual meeting of The Bioelectromagnetics Society, San Antonio, TX, June 10-14, 1990.

11. D. Krause, W.J. Skowronski, J.M. Mullins, R.M. Nardone, and J.J. Greene, *Selective enhancement of gene expression by 60-Hz electromagnetic radiation*, presented at the Bioelectrical Repair and Growth Society Meeting, Philadelphia, PA, October 1990.

12. D. Krause, J.M. Mullins and T.A. Litovitz, *Effect of coherence time of the applied field on the bioelectromagnetic enhancement of ornithine decarboxylase specific activity*, presented at The Annual Review of Research on Biological Effects of 50 and 60 Hz Electric and Magnetic Fields, sponsored by The Department of Energy, American Public Power Assoc. and the Edison Electric Institute, Denver, CO, November 1990.

13. T.A. Litovitz, *On the effect of low level electromagnetic fields on chick embryos*, invited paper presented at the 12th Annual International Conference IEEE Engineering in Medicine and Biology Society, March 1991.

14. T.A. Litovitz, C.J. Montrose and W. Wang, *Dose response implications of the transient nature of the bio-effects of electromagnetic fields*, presented at the EPRI workshop on dosimetry, Carmel, CA, March 19-22, 1991.

15. T.A. Litovitz, *Biological response to weak ELF electromagnetic fields*, presented at the AAPT Symposium on Low-Level Electromagnetic Radiation on Living Systems at the American Physical Society Meeting, Washington, DC, April 25, 1991.

16. C.J. Montrose and T.A. Litovitz, *A proposal to account for the unusual dose-response characteristics of systems exposed to EM fields*, presented at the 13th annual meeting of The Bioelectromagnetics Society, Salt Lake City, Utah, June 23-27, 1991.

17. T.A. Litovitz, C.J. Montrose and T.L. Litovitz, *On a genetic predisposition to EMF-induced effects in chick embryos*, presented at the 13th annual meeting of The Bioelectromagnetics Society, Salt Lake City, Utah, June 23-27, 1991.

18. D. Krause, T.A. Litovitz, J.M. Mullins, R. Nardone and J.J. Greene, *Effect of ELF signal coherence time on ornithine decarboxylase activity and mRNA transcription,* presented at the 13th annual meeting of The Bioelectromagnetics Society, Salt Lake City, Utah, June 23-27, 1991.

19. T.A. Litovitz, C.J. Montrose and T.L. Litovitz, *On the adverse effect of electromagnetic fields,* presented at the International Conference on Environmental and Industrial Toxicology: Research and Applications, July 22-26, 1991, Bangkok, Thailand.

20. J.M. Mullins, D. Krause, A. Desta and R.M. Nardone, *Exposure parameters affecting extremely low frequency electromagnetic field enhancement of ornithine decarboxylase activity in mammalian cultured cells,* presented at The Annual Review of Research on Biological Effects of 50 and 60 Hz Electric and Magnetic Fields, sponsored by The Department of Energy, the Edison Electric Institute, the American Public Power of Assoc. and Wisconsin Utilities Association, Milwaukee, WI, November 4-8, 1991.

21. K.M. Brown, P.G. Doinov, M.K. Barber, T.L. Litovitz and T.A. Litovitz, *Effects of extremely low frequency electromagnetic fields on chicken embryogenesis,* presented at the 1992 Annual Meeting of the Society of Toxicology, Seattle, WA, February 23-27, 1992.

22. T.A. Litovitz, C.J. Montrose and P. Doinov *Spatial and temporal coherence affects the response of biological systems to electromagnetic fields,* presented at the First World Congress on Bioelectromagnetism, Orlando, Florida, June 14-18, 1992.

23. J.M. Mullins, D. Krause and T.A. Litovitz, *Simultaneous application of a spatial ᵗʰ⸱ coherent noise field blocks the response of cell cultures to a 60-Hz electromagnetic field,* presented at the First World Congress on Bioelectromagnetism, Orlando, Florida, June 14-18, 1992.

24. J.M. Farrell, M. Barber, P. Doinov, D. Krause and T.A. Litovitz, *Effect of 60-Hz sinusoidal magnetic fields on ornithine ecarboxylase activity in developing chick embryos,* presented at the First World Congress on Bioelectromagnetism, Orlando, Florida, June 14-18, 1992.

DOCTORAL THESES PUBLISHED

1. B. Saif, *Dielectric relaxation in aqueous DNA solutions,* Catholic University of America, Physics Department, May 1990.

2. L.M. Penafiel, *Dielectric properties of polyelectrolytes: Mechanisms of high-frequency dispersions,* Catholic University of America, Electrical Engineering Department, May 1991.